
QUARTERLY REPORT

RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES, NAVY OPERATIONAL- RELATED INJURIES AND ILLNESSES AND APPROACHES TO ENHANCE NAVY/MARINE CORPS PERSONNEL COMBAT PERFORMANCE

Prepared for

Naval Medical Research Institute
Bethesda, Maryland 20814

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Prepared by
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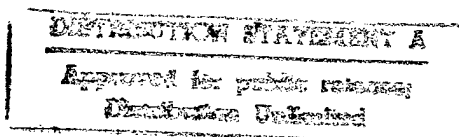


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QUARTERLY PROGRESS REPORT
OPTION YEAR ONE
GC-PR-2728-00

CONTRACT NUMBER: N00014-95-D-0048

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**RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES,
NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND
APPROACHES TO ENHANCED NAVY/MARINE CORPS PERSONNEL
COMBAT PERFORMANCE**

I. INTRODUCTION

This report summarizes the results of GEO-CENTERS' technical activities for the first option year one of the Naval Medical Research Institute (NMRI) Contract N00014-95-D-0048, Delivery Orders 002, 003 and 004. The delivery orders encompass a variety of scientific studies that are capable of supporting ongoing and projected programs under the cognizance of NMRI; NMRI TOX/DET-Dayton, OH; NMRI/DET-San Antonio, TX; NDRI-Great Lakes, IL; the NDRI Detachment-Bethesda, MD; the National Naval Medical Center-Bethesda, MD; and the U.S. Navy Clothing and Textile Facility-Natick, MA.

The format for these periodic technical progress reports consists of four sections each listed by the location of the research. The sections are (1) Descriptions of work to be performed, (2) Objectives planned for the current reporting period, (3) Summary of work performed during current reporting period, and (4) Objectives for the next reporting period. Accumulated scientific reports, technical reports and journal articles are being provided as part of this quarterly technical progress report. Specifically, the research conducted by GEO-CENTERS during this quarterly reporting period has been focused on the following general scientific programs:

- A. Infectious disease threat assessment and preventive medicine programs.
- B. Immune cell biology, wound repair and artificial blood studies.
- C. Biomedical diving programs.
- D. Personnel performance enhancement programs.
- E. Breast Care Center.
- F. Directed Energy Effects Research
- G. Dental related diseases.
- H. Toxicological studies.
- I. Human Performance and U.S. Navy Clothing Development

DATA QUALITY IMPROVED 4

II. NMRI, Bethesda, MD

A. INFECTIOUS DISEASE THREAT ASSESSMENT AND PREVENTIVE MEDICINE PROGRAMS

DESCRIPTION OF WORK TO BE PERFORMED

Fernando

- Performs research on the molecular detection of orthopox viruses, which can be a warfare as well as an epidemiological threat. The research involves the design and optimization of DNA probes and tests that will identify and differentiate orthopox virus genomic profiles, finally proceeding to the diagnostic validation of these tests. These tests fall into three levels, namely, primary screening, secondary confirmatory and tertiary characterization.

Jendrek

- Conducts fermentations in a BL-3 suite and depending on the organism of the fermentation may also perform some or all of the downstream processing associated with the project. He also creates all associated paperwork (standard operating procedures, batch records, etc.) with the fermenter and related equipment. Scott also does much of the HPLC work towards optimizing current protein purification methods and procedures, as well as some of the Molecular Biology associated with his position.

Kerby

- Senior Scientist; develop diagnostic systems to detect and differentiate Orthopoxviruses.

Christian, Wohlrabe

- Provide assistance to the adenovirus surveillance project within the Preventive Medicine Division of Naval Hospital, Great Lakes, IL.
- Assist in the development and implementation of "Operation Stop Cough", a programmatic approach to reducing respiratory illness among Navy recruits.



Heavey

- Senior Scientist II

Mihalic

- Serve as an entry level lab technician in developing and optimizing electrophoretic assays applicable to the overall interests of the division.

Weeks

- Serve as an associate of the principal investigator for a research program involving pathogenic, molecular, and biochemical analysis of bacteria and their virulence factors. Experimentation requires knowledge and proficiency of laboratory techniques and procedures for performing biochemical and immunological analyses. Conducts surveys of the scientific literature to develop background data on techniques and formulates approaches for the investigations, develops experimental protocols, defines the objectives and priorities of subsidiary problems and arranges the details of cooperative investigations with other organizations when necessary. Is responsible for the general administration of the laboratory reagents, solutions, enzymes, and other materials and equipment used in conducting the studies described. Is responsible for the cleanliness and orderliness of working areas, freezers, and refrigerators. Is responsible for the training and orientation of all new laboratory technicians. Organizes and accumulates repositories of bacterial strains, plasmids, enzymes and sera with sufficient documentation of the histories of each. Maintains sufficient stocks of all reagents, supplies, and equipment required for a well organized molecular biology laboratory. Performs other duties as assigned. Immunizations are required.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Fernando

- Continue work with PCR on new strains of the orthopox virus. The RFLP profiles of the four segments on the new strains will be completed. If amplification problems occur with the new strains, the primers will be modified with inosine or universal





GEO-CENTERS, INC.

October 6, 1997

Commanding Officer
Naval Medical Research Institute
8901 Wisconsin Avenue
Bethesda, MD 20814-5044

Attention: Lt. Tracy Theroux

Reference: N00014-95-D-0048
GC-2728/3078/3117

Dear Lt. Theroux:

In accordance with the Contract Data Requirements List (DD Form 1423), ELIN A001, GEO-CENTERS, INC. is pleased to submit its quarterly progress report for the period June - August, 1997.

If you have any questions, please contact Dr. Monty Herron at (301) 231-6144 or the undersigned at (617) 964-7070.

Sincerely,

Robert P. Hallsworth
Contracts Administrator

Enclosure

cc: NRL/Code 2627 - 1 copy
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base and re-evaluate. Begin evaluating the primers designed by Sofi Ibrahim for the remaining segments. Begin generating RFLP profiles for the new segments with all orthopox viral strains available. If necessary, modify the latter primers as mentioned before. As primer/target mismatches for different strains are expected address these problems by modification or new designs of primers. If all the segments are not easily amplifiable in every strain and if the CDC cannot provide positive primers, make new designs on shorter (10-15 Kbp) segments, particularly for the outer segments which are likely to be the main problem. Complete DENDRON analysis on available RFLP data and start developing a database. Protocols will also be completed this quarter.

Jendrek

- Scott will perform another fermentation to test the HIC capacity and protein loss vs. the HQ step, this fermentation will also be carried +2 hours from the normal harvest time to see if the HIC can eliminate the contaminants usually associated with the extended fermentation time. Scott will grow the F1-V producing *E. coli*, if the strains become ready within the next quarter (Note : These strains DID NOT become available). He will develop a growth protocol testing various media and media supplements. After Scott has determined optimal growth conditions, this will be scaled up to the 20 liter level. If those strains are not ready, Scott will grow LF and EF producing strains at the 100 liter level. He will then purify those individual proteins and create a protocol for those strains.

Kerby

- Develop the primers, from my database of sequences, that will be used in the TaqMan Diagnostic System.
- Clone the seven genes of Variola by Zero blunt cloning the PCR products obtained from Dr. Esposito of CDC in Atlantic.
- Start cloning at the seven genes of interest from Vaccinia, Camelpox, Monkeypox, and Cowpox viruses.
- Analyze all sequences and develop primers that can be used in Rapid PCR diagnostics



Christian, Wohlrabe

- Continue culture surveillance for adenoviral illness among recruits.
- Assess ways to augment culture returns from our outpatient clinics.
- Assist with an evaluation of vaccine status and MMR immunity among new recruits.
- Collect metrics on hygiene and hand washing as part of the needs analysis of Operation Stop Cough.
- Continue to liaison between investigators, providers, laboratory staff, and patients to provide adenovirus surveillance information.

Heavey

Orthopox Project

- Test existing anti-vaccinia Mabs for their reactivity with various orthopoxviruses to determine if any candidate Mabs exist that would be useful as a reagent for detection of orthopoxviruses in an aerosol.
- Clone gene from the more distantly related monkeypox virus, which may prove useful as targets for detection of orthopoxviruses in aerosols.

Filovirus Project

- Continue work on identification of protective epitopes of Marburg virus.
- Specifically, challenge animals that have already been immunized with VEE replicon-based vaccine.

Mihalic

- Make our SDS-PAGE and western blots quantitative, as well as qualitative assays by learning to use a software package combined with a scanner to measure protein levels and quantitatively calculate molecular weight.
- Finish the research for a poster presentation at the Tropical Medicine meeting this December.
- Assist in developing a human IgM western blot against Dengue virus.
- Optimize the purification of the recombinant hantaan antigen and do purity and activity analyses on it.
- Learn more about HPLC and its applications to my work.



Weeks

- The first objective of this quarter is to finish the mapping of Mud 1 mutants for possible genes in the pFra plasmid of *Yersinia pestis*. This is the largest plasmid in this bacterium, but very little is known about the genes of this plasmid. It is known that this plasmid possesses the F1 capsular antigen of the bacteria, which is activated at 37oC (mammalian hosts). The only other gene on this plasmid is the Mouse Toxin (MT), which is not of interest to the research. Because of the above facts, the sequencing of any new genes on this plasmid will be of great scientific interest to all researchers in this field.
- The second objective for this quarter is to continue the binding assays of formyl-Met-Leu-Phe (fMLP) to human polymorphonuclear neutrophils to evaluate the activity of V antigen in *Yersinia pestis*. It is believed that this antigen is paramount to the survival mechanisms of *Yersinia pestis*. This antigen is supposedly released by the bacterium at first contact with a human host. This antigen proceeds to debilitate the immune system of the host by first, preventing the inflammatory response, which is the first line of defense in the human body. This antigen is also thought to inactivate complement, another line of immunological defense. These experiments will attempt to answer the questions of whether V antigen actually prevents an inflammatory response, and how it accomplishes this. Various other factors will also be measured, including superoxide production, release of interleukins, and cytoskeleton rearrangement by polymorphonuclear neutrophils.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Fernando

- The RFLP profiles of the four segments on the new strains were completed. The primers designed by Sofi Ibrahim for the remaining segments were evaluated. DENDRON analysis on available RFLP data was completed and the development of a database was initiated.

Manuscripts

- A finalized manuscript has been sent for government clearance.

Jendrek

- Scott completed the development of the hydrophobic interaction chromatography (HIC) step for the further purification of Protective Antigen (PA) from *Bacillus anthracis*. The group performed a fermentation to test the HIC step in place of the HQ step of the purification, the resulting PA was of a much higher quality. Two fermentations were run with extended hours to see how the production of PA was affected by the longer fermentation times and to determine the amount of protease degradation. Scott has begun work on the LF producing strains of *B. anthracis* and is currently working on media optimization for that strain. Scott also started work on the purification of the isoforms of Protective Antigen by using a Resource Q column from Pharmacia, he will continue this work into the next quarter when the new HPLC has arrived.

Publication

- A publication is being circulated for internal review, it has not been sent to any journals as of yet.

Kerby

- All the sequencing primers were analyzed and results given to Dr. Ibrahim for possible probe development in the TaqMan Diagnostic System.
- Five of the seven genes of Variola were successfully cloned and validated by sequence and restriction endonuclease analysis and glycerol stocks given to Dr. Gilligan for further development.
- The Thymidine Kinase gene for Vaccinia, Camelpox, Monkeypox, and Cowpox were cloned by the TA cloning vector system and glycerol stocks made.
- analyzed PCR products and cloned fragments for other projects, such as Hantaan virus S segment encoding nucleocapsid protein, Dengue clones, Ebola clones, and various anthrax fragments.
- Also helped in the training of two new DSD staff members and four summer students.

Christian, Wohlrabe

- Continued to distribute, collect, and arrange mail-out of culture materials for adenovirus surveillance. Provided astute quality control on culture media.
- Provided assistance to medical professionals in obtaining adenovirus cultures.
- Reviewed records and provided quality control for case reports of respiratory illness.



- Inspected hand washing facilities for recruits, as a quality metric for Operation Stop Cough.
- Collected and analyzed recruit survey data on hand washing and past MMR vaccination.
- Completed quarterly safety training and "environment of care" assessment; assisted in preparation for JCAHO inspection.
- Assisted with arranging an independent evaluation of SHIP, the recruit inprocessing medical questionnaire.

Heavey

Orthopox Project

- An existing battery of 90 murine Mabs were tested for the capacity to bind not only vaccinia virus, but cowpox and monkeypox viruses. Several non-redundant candidate Mabs that cross-reacted well with cowpox and with the more distantly related monkeypox virus were identified.
- An existing battery of 70 human anti-vaccinia mabs (Fabs) were tested for the capacity to bind both vaccinia and monkeypox virus. Several non-redundant candidate Mabs were identified that cross-reacted well with the more distantly related monkeypox virus.
- For epitope mapping purposes, several vaccinia virus and monkeypox virus genes have been cloned, expressed in vitro, and confirmed to be immunoreactive.
- Cloned several genes from monkeypox virus that may be useful as targets for detection of orthopoxviruses in aerosols. These include the monkeypox genes for the vaccinia homologues of: D8L, L1R, A33R, A34R, and H3L. Cloned genes were confirmed to express product in vitro.

Filovirus Project

- Continued guinea pig challenge experiments to determine the efficacy of VEE replicon-based vaccines for Marburg virus (MBGV). Guinea pigs previously immunized with VEE replicon which exp

Mihalic

- Received the scanner and software for gel analysis and can do quantitative SDS-PAGE.
- Have helped the labs by bringing another assay that can help in analyzing our diagnostic tools. The research for the poster is almost finished, presently testing the assay against a test panel of human sera to confirm it as a diagnostic assay.
- Because of the priority of the various Hantaan projects in the lab, the project involving the Dengue virus has been delayed and a lot of work has been done on the optimization of the recombinant hantaan antigen. Although we are able to purify it through metal chelating HPLC, we are not happy with the yield and our trying to optimize the induction of the recombinant.

Presentations, abstracts, etc.

- "Production and Purification of a Recombinant Hantaan Nucleocapsid Protein and Its Application in diagnostic Assays." Moss, DW, Courtney, BC, Fledser, DM, Mihalic, KA, Rossi, CA, Kerby, SB, Phillips, SM, and Henchal, EA.; Diagnostic Systems Division, U.S. Army Medical Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, MD 21702-5011.
- "Purification of chicken IgY Anti-Eastern equine Encephalitis virus and adsorption of cross reactivity by Immunoaffinity HPLC" Moss, D.W., Mihalic, K.A., Rossi, C.w., Parker, R.W., diagnostic systems Division, USAMRIID, Fort Detrick, MD 21702-5011.

Weeks

- Work was started on the binding of fMLP to human polymorphonuclear neutrophils. Although many problems were encountered during the start of this work, they have been dealt with, and the project is proceeding as planned.

Publications

- V Antigen of *Yersinia pestis* Inhibits Neutrophil Chemotaxis. S. Welkos, A. Friedlander, D. McDowell, **J. Weeks**, and S. Tobery. Poster Abstract for American Society of Microbiology meeting in Miami, May 1997.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Fernando

- N/A

Jendrek

- Scott will continue the work with the LF producing strain of *B. anthracis* and then scale that up to the 20 liter level. Scott will then create a purification protocol for LF. When the new HPLC comes in, Scott will perform more of the isoform separations using the Resource Q resin.

Kerby

- Finish the cloning of the other six genes of interest for Vaccinia, Camelpox, Monkeypox and Cowpox viruses.
- Assist in the development of probes for the TaqMan system.
- continue the support of other programs for sequencing diagnostics and verification of PCR products and clones.

Christian, Wohlrabe

- Continue work on the adenovirus surveillance project, modifying procedures as the protocol changes and recruit needs change.
- Continue to provide data on hygiene and hand washing, as metrics for Operation Stop cough.
- Re-evaluate development of video aids to encourage hygiene/hand washing among recruits
- Re-evaluate recruit surveys in late-training, to assess the relationship between hygiene behaviors and lost-time for respiratory illness.
- Enter and analyze data on past vaccination/MMR immunity among new recruits.

Heavey

Orthopoxvirus Project

- Confirm sequences and transfer the cloned monkeypox virus genes into a VEE replicon for expression in vitro, and confirm expression of proteins in vitro.

- Initiate efforts to obtain variola genes through WHO, which may prove as useful targets for detection of orthopoxviruses in aerosols.

Filovirus Project

- Evaluate post-challenge serum from guinea pigs which were immunized with VEE replicon expressing MBGV proteins via ELISA to determine if sterile immunity was obtained.
- Immunize a new group of guinea pigs with VEE replicons to examine the ability of individual gene products to protect against a heterologous challenge virus. Specifically, MBGV GP, NP, and VP35 will be used to immunize animals three times at 28 day intervals. After completion of immunization schedule, half the animals will be challenged with a homologous virus isolate and half with a heterologous virus isolate.

Mihalic

- Conclude my research for my poster and hand over the written material and data to the visual arts department to be mounted on poster board.
- Aid in the recovery and purification of a recombinant hantaan nucleocapsid protein.
- Now that hantaan western blot project is winding down, begin the dengue project and have an IgM western blot protocol established.
- Incorporate the advantages of the scanner software to analyze our results and make SDS-PAGE and western blots a more useful tool for us.

Weeks

- The objectives for next quarter are to finish the mapping of possible F1 genes in pFra and to produce more data on the binding of fMLP to human polymorphonuclear neutrophils.



II. NMRI, Bethesda, MD

B. IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND ARTIFICIAL BLOOD PROGRAM

DESCRIPTION OF WORK TO BE PERFORMED

Bitensky

- Principal Investigator who provides technical direction for the Geo-Centers/NMRI blood storage contract. The other scientists on the project, including Dr. Yoshida, respond to Dr. Bitensky, who is a senior research professor in the Boston University college of Engineering

Chavez

- Principal Investigator, Blood Research Detachment - I perform basic research on the physical properties of hemoglobin and erythrocytes. Hemoglobin is the protein within the erythrocyte (red blood cells) responsible for oxygen uptake and transport. The projects listed below are being initiated to study the red cell aging problem in hopes of extending the shelf life of blood, a primary mission goal. Blood is a valuable resource to the U.S. Army. Currently, due to remote locales, transportation, storage requirements, etc., a modest increase in the shelf life of blood would be highly beneficial for the military both in logistics and cost savings. A thorough understanding of erythrocyte structure and function would provide the knowledge base required to impede or prevent oxidative damage, cell degradation and rigidity, thus extending the shelf life by preserving erythrocyte viability, integrity, and function. Hemoglobin oxidation, heme stability within hemoglobin, and nitric oxide interaction with the erythrocyte are the major focus areas at this time.

Christensen

- Richard Christensen is a Scientist I and representative for GEO-CENTERS for the NMRI contract. He serves as and performs work as a research assistant. He is responsible for implementing and carrying out aspects of the Navy Blood Storage Project being conducted at the Boston University. Mr. Christensen responds to Tatsuro Yoshida, Ph.D. and Mark Bitensky, M.D., both whom serve as investigators of the project.



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Gabel

- Chris Gabel is a researcher for Geo-Centers for NMRDC . He works as a research assistant under Dr. Tatsuro Yoshida and Dr. Mark Bitensky at the Boston University Department of Biomedical Engineering. Chris is helping to create an in vitro test for red blood cells that will use microfabricated structures to determine the physical health of red blood cells.

Gordon

- Scientist I and representative for GEO-CENTERS for the NMRI contract. She serves as and performs work as a research assistant. She is responsible for implementing and carrying out aspects of the Navy Blood Storage Project being conducted at the Boston University. Miss. Gordon responds to Tatsuro Yoshida, Ph.D and Mark Bitensky, M.D., both whom serve as investigators of the project.

Lee

- Scientist I and representative for GEO-CENTERS for the NMRI contract. He serves as and performs work as a research assistant. He is responsible for implementing and carrying out aspects of the Navy Blood Storage Project being conducted at the University of New Mexico (UNM). Mr. Lee responds to Dr. Tatsuro Yoshida and Dr. Mark Bitensky, both of Boston University, whom serve as investigators of the project

Mark

- Scientist I and representative for GEO-CENTERS for the NMRI contract. She serves as and performs work as a research assistant. She is responsible for implementing and carrying out aspects of the Navy Blood Storage Project being conducted at Boston University. Miss Mark responds to Tatsuro Yoshida, Ph.D. and Mark Bitensky, M.D., both who serve as investigators of the project

Tarr

- Senior Research Scientist for GEO-CENTERS for the NMRI contract. He serves as a protein chemist/biochemist working on the Navy Blood Storage Project begin conducted at Boston University. His main duty is to design, coordinate and supervise experiments in



chemical modification of the red cell surface and analysis of the cell membrane and associated proteins. George Tarr reports to Dr. Mark Bitensky at Boston University.

Thomas

- Engineer III, serves as the Computer Aided Design Drafter(CADD) Manager, representing GEO-Centers, Inc., in support of biomedical research and development activities located at the Walter Reed Army Institute of Research(WRAIR)-Health Facility Planning Agency(HFPA) Office. He is responsible for organizing a CADD department, managing all aspects of computer-aided design and systems management for all CADD workstations. Mr. Thomas is also responsible for implementing procedures for manipulation of drawing files and developing user(working) drawings from existing documentation of new health facility.

Yoshida

- Part time Senior Scientist and representative for GEO-CENTERS for the NMRI contract. Her serves as a biochemist /red cell physiologist working on the Navy Blood Storage Project being conducted at Boston University. His main duty is to design, coordinate and supervise experiments carried out by GEO-CENTERS scientists on the blood storage project at Boston University and at the University of New Mexico with Dr. Bitensky. Tatsuro reports to Drs. Mark Bitensky at Boston University and Monty Herron at Geo-Centers.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Bitensky

- During this period the principal focus has been to understand and address the discrepancies between our in vitro survival measurements and the expected performance of our red cell in vivo human survival studies. Since our in vivo data raise expectations that the oxygenated red blood cells should show excellent properties including in vivo survivals following 18-21 weeks of refrigerated storage, and since such expectations are strongly demonstrated by the in vitro battery of tests, we are understandably disappointed that we have managed only to prolong our storage when referenced by in vivo survivals from the conventional 6 to our current best effort of 9-10 weeks. This discrepancy, we believe, reflects the fact during prolonged refrigerated storage, several problems arise which interfere with the red cells' circulating functions in vivo. These problems are associated with a change in red cell ionic composition and water content, changes in cytosolic ph, and changes in the composition acid composition, and ph of the backing medium. We are focusing on developing exchange solutions to correct the changes that occur to the storage solution, as well as changes in the storage solution which are anticipated to interfere with the accumulation of problems during storage. These changes include changing the amount of impermeant ion species in the



storage bag, as well as introducing additional amounts of sodium In the storage bag, and small quantities of amonium ion. We are also developing a new microfabricated array which is instrumented by a computer on a silicon ship, and which behaves as a surrogate capillary. In order to evaluate the elusive parameter of red cell deformability as part of our surrogate battery of tests which we hope will eventually obviate the need for in vivo survival measurements.

Chavez

- Continuation of the nitric oxide - hemoglobin binding experiments
- completion of the calorimetry experiments
- Initiation of sulfhydryl reactivity studies of the red cell membrane as a function of aging

Christensen

- Three variables were evaluated for the efficacy of each in improving the viability of blood stored at 4°C for more than six weeks:
 - Type of storage solution: OFAS1 or AS3
 - Hematocrit
 - Presence of absence of oxygen

Gabel

- The main objective for this quarter was to create and implement microfabricate devices which test the physical health of red blood cells stored under various conditions. Red blood cells are observed as they deform around and move through these microstructures. The goal was to first implement a simple design for the microstructures and then optimize this design as an in vitro test for stored red blood cells. In addition, a number of tests were to be done on healthy, as well as, specifically altered red blood cells using the micro-structures.

Gordon

- The initial objective for the quarter was to outfit the laboratory with microbiology supplies and equipment needed to perform bacteriological assays on stored blood. Yersinia enterocolitica has been reported as being present in stored blood and as the cause of endotoxic shock in some blood recipients. The primary objective, therefore, is to develop assays that could detect the presence of Yersinia in stored blood and to



monitor the progression of this organism's growth throughout the extended blood storage period.

Lee

- The main objective of this quarter was to complete two in vivo studies begun in April and May. The first study is designed to study the effects of anaerobically stored blood using Nutricel AS3 at a hematocrit of 60. The second study examines blood stored anaerobically in our new blood additive (OFAS 1) at a hematocrit of 30 (down from 40 in previous in vivo study). A total of 24 subjects were to be used for these studies.
- Another objective was to study, by in vitro methods, blood storage in a oxygen rich environment and in a carbon monoxide rich environment, work previously done by Drs. Bitensky and Yoshida.
- The third objective was to analyze the data from the in vivo studies.

Mark

- The primary objective for the reporting period has been to determine the phosphorylation levels of the cytoskeleton of the red blood cell (RBC) in the capillaries. This laboratory has found evidence using a micro-array that simulates the capillaries and allows one to evaluate the red blood cell, that the missing link in understanding how to store the RBCs is in the deformation of the cytoskeleton. RBCs that have been stored for 12 weeks are not able to deform and circulate through the capillaries as well as fresh blood. In order to deform through the capillaries, it has been hypothesized that certain proteins in the cytoskeleton such as spectrin and band-3 are phosphorylated. The phosphorylation adds negative charges to amino acids in the protein which repel and allow the protein to deform. This results in the deformation of the red blood cell. Therefore the ability to store blood may result from the ability to understand the deformation due to phosphorylation of the red blood cell.

Tarr

- The main objectives of this quarter included the evaluation of several strategies for chemical modification of the red cell surface that might be used to stabilize the quantity and arrangement of components, thus improving in vivo survival of stored cells. A second objective was to evaluate the use of enzymatic digestion and mass spectrometry for the analysis of crude preparations of cytoskeleton in order to extend our capacity for in vitro evaluation of red cell storage conditions.



Thomas

- Develop user drawings of first, second, third and penthouse floors of new health facility.
- Provide assistance to COE investigating all existing drawing documentation for current modifications.

Yoshida

- The main objectives of this quarter included completing the second in vivo 24 hr survival testing at the University of New Mexico, establishing procedures to evaluate rheological properties of stored cells, devising procedures to restore deformability of red cells after prolonged storage, and examine the growth of contaminant micro-organisms under prolonged anaerobic storage conditions.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Bitensky

- The principal focus during this last quarter has been to compare in vivo and in vitro diagnostics over periods of 10 weeks of storage and beyond, to develop the filter and array technologies that would permit us to measure red cell deform ability, and to compose and evaluate a series of storage solutions in which sodium ion and mannitol vary. We are also looking at regeneration solutions.

Chavez

- The manuscript "An Improved Process for the Production of Sterile Hemoglobin solutions" is in press.
- The manuscript "Liposome Encapsulation Attenuates Hemoglobin-Induced Vasoconstriction in Rabbit Arterial Segment" has been published in the Journal of Applied Physiology.
- Calorimetry experiments on the step-wise ligation of hemoglobin are still proceeding. Barring any more setbacks, completion will be accomplished in the coming quarter.
- Two summer students, Jamie Fan and Jennifer Nguyen, participated in WRAIR's Science and Engineering Apprentice Program. The majority of their work involved



getting the nitric oxide analyzer on-line. This apparatus can measure the amount of nitric oxide accurately to picomole amounts, a necessity in quantifying formation of nitrosothiol on the red cell membrane. Preliminary results indeed show a significant amount of nitrosothiol formation on the membrane when the red cells are exposed to nitric oxide. This result confirms the hypothesis that thiols on the red cell membrane can compete favorably with hemoglobin for the nitric oxide. Several titrations and experimental conditions need to be performed in order to confirm this finding, as our result contrasts other studies which claim that significant nitrosothiol formation occurs intracellularly on the hemoglobin. On a clinical basis, this may impact critical areas in trauma care such as oxygen transport and delivery, ischemia, and blood pressure regulation. In addition, these findings may help further the understanding the function of red cell membrane proteins and lead to improved storage media.

- As part of the blood storage program, the reactivity of sulfhydryl(thiol) groups on the red cell membrane are being measured as a function of storage time. Other studies suggest that oxidation of the thiol group on the red cell membrane proteins results in crosslinking of the protein, increasing rigidity of the red cell, decreasing functionality of the protein, and potentially serving as a marker for phagocytosis. In conjunction with other assays being performed, the measurement of sulfhydryl reactivity as a function of storage time is currently being done for possible correlation between the reactivity of sulfhydryl groups and parameter such as cell lysis, phagocytosis, cell fragility, and in vivo survivability.

Publications, Abstracts, etc.

1. "An Improved Process for the Production of Sterile Modified Hemoglobin Solutions" F.A. Highsmith², C.M. Driscoll², B.C. Chung², M.D. Chávez¹, V.W. Macdonald¹, J.M. Manning³, L.E. Lippert², R.L. Berger², and J.R. Hess², Biologicals, in press.

¹Blood Research Detachment, Walter Reed Army Institute of Research; ²Bionetics Corporation, Rockville, MD; ³The Rockefeller University, New York, NY.

2. "Liposome Encapsulation attenuates Hemoglobin-Induced Vasoconstriction in Rabbit Arterial Segment" A.S. Rudolph¹, A. Sulpizio², P. Hieble², V. M. Macdonald³, M.D. Chavez³, and G. Feuerstein², J Appl Physiol 1997, 82(6), 1826-1835.

¹Center for Bio/Molecular Science and Engineering, Code 6910, Naval Research Laboratory, Washington, D.C. 20375-5348; ²Dept. of Cardiovascular Pharmacology, SmithKline Beecham, King of Prussia, PA 19406; ³Blood Research Detachment, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100



Christensen

- Three groups of blood were stored and evaluated during 15 week periods. Samples were assayed on a weekly or bi-weekly schedule for the following in vitro parameters: lactate, ATP, hemolysis, and vessicle formation. In addition, mean cellular volume (MCV) was measured at intermittent periods during storage.
- Data obtained from these storage sets indicated that blood stored in OFAS1 showed improved viability as predicted from all in vitro parameters. However, MCV was shown to be smaller in blood stored in OFAS1 than in AS3, the storage solution used currently by blood banks. Decreased MCV may adversely affect viability due to a decreased deformability of stored cells, but this has not been thoroughly evaluated.
- Anaerobically stored blood showed only slightly better in vitro parameters than identical blood stored in air. In contrast, blood stored at decreased hematocrits showed marked improvement in all in vitro diagnostics.

Gabel

- A number of different designs for the microstructure devices have been made (at the Cornell Nanofabrication Facility) and used in experiments. The behavior of healthy blood cells in these micro-arrays has been well established. This includes flow rate of the cells through the microstructures, as well as the extent of their deformability. In addition, a number of experiments have been run that studied the physical behavior of red blood cells after specific alterations (such as changes in osmotic pressure, temperature, exposure to chemicals etc...).

Gordon

- Work during this quarter included the purchase of an incubator, an environmental shaker, petri dishes, test tubes and racks, bacteria incinerator, etc. and representative cultures (used for preliminary assay design) of Yersinia enterocolitica and a few organisms known to be responsible for septicemia. Once all supplies and equipment had been set up, Miss Gordon began outlining the growth phases and parameters of the various bacteria. A number of assays were initially performed until the most precise method (pour plate) was defined. In addition, an extensive literature search was conducted on stored blood contaminants and blood born microbes that cause septicemia in hospital patients.



Mark

- During this reporting quarter Julia Mark has performed an extensive literature search on this topic and she has determined experimental protocols which would be appropriate to carry out the above objectives. She determined that isolating the red blood cells from the capillaries, freezing them in a kinase/phosphatase buffer, isolating the proteins in the cytoskeleton, and running the proteins on a 2-D electrophoresis gel would be the best method to carry out the project. Single changes in phosphorylation of proteins are visible on 2-D gels. She has gained experience performing the 2-D gel protocol and has become proficient in the technique. She has also isolated the cytoskeleton from red blood cells and run them on the 2-D gels to determine the pattern of the cytoskeleton proteins on the gel.

Lee

- Six of twelve subjects completed the study on anaerobic AS3 blood. It was decided, based on these results, that the remaining six units would not be drawn, as no useful data could have come from them. Eleven of twelve subjects completed the second study on OFAS 1, Hct=30 blood.
- Data from these in vivo survival studies were analyzed. Based on this analysis as well as data from in vitro studies done by Dr. Yoshida at Boston University, it was decided that a lower hematocrit of 30 was not beneficial to the survival of red cells in vivo and changes to protocols will be made to reflect this analysis.
- Studies evaluating carbon monoxide and oxygen rich environments were completed. Data confirmed previous data obtained at Los Alamos and will be used to prepare manuscripts for publication in a professional journal.

Tarr

- Several procedures for covalently attaching acidic carbohydrates to model proteins were explored in terms of gentleness of conditions (compatible with red cell physiology), degree and site of modification and physical and biochemical properties of the product. While all of these procedures were found to be potentially usable, glaciation by Schiff-base formation followed by cyanoborohydride reduction (to stabilize) seemed most effective, especially if rapid and extensive modification is the desired result.
- Initial tests of mass spectrometric analysis of digests of crude cytoskeletal preparations were promising, but difficult to interpret. A database of structural



information, especially sequences and post-translation modifications, of all of the components of the cytoskeleton is being assembled. This information is available from extensive on-line databases (which themselves are far too large for effective use) and reasonably complete, and should help considerably in the assignment of signals to particular proteins and regions thereof.

Thomas

- Attended MicroStation 95 conference/seminar to provide further efficiency for production of working drawings.
- Assisted COE in investigating all existing drawing documentation for current modifications.
- Generated drawings of each floor, for use in guided tours of new facility.
- Generated color coded drawings which identified general departments of each floor.
- Generated user ground floor drawings for designated occupied spaces of Navy Diving Research.
- Generated general occupancy plan drawings for 1st floor renovations of Sec. B.
- Generate drawings for Senate meeting, showing specific lab assignment designations of each floor.
- Coordinated with Architectural firm contractors to obtain and plot drawings of designated spaces and space assignments of each floor.
- Aided in maintenance of Construction Management System (CMS) database.

Yoshida

- The second *in vivo* 24 hr survival testing at the University of New Mexico was completed and *in vitro* diagnostic parameters were determined. The reduced storage hematocrit with OFASI was determined to have a slightly negative effect on *in vivo* survival. This result was correlated with the reduced deformability as determined by the filtration assay.
- The other cohorts were stored for 9 weeks under anaerobic conditions using a conventional additive solution AS3. This test was conducted to assess the effect of oxygen removal on otherwise current blood-banking condition, and the survival rate was determined to be 74%. Although significant improvements in survival rate over the aerobic storage were observed, it was a less than acceptable 75%. From these results and experiments carried out *in vitro*, we believe that reduction of the storage hematocrit from 70% (currently used at the blood banks) to 40% would permit an acceptable 9-11 weeks of anaerobic storage using an AS3 additive solution.



GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Bitensky

- During this coming reporting period, our principal focus is to yet further improve our abilities to evaluate the microscopic and molecular conditions of the cytoskeleton and lipid layers following various storage periods. In addition, we are focusing intensely on our abilities to measure red cell deformability and our abilities to deoxygenate our red cell storage system.

Chavez

- Continuation of the nitric oxide experiments with erythrocytes - Nitric oxide is known to cause vasodilation. Nitrosothiols, formed between nitric oxide and thiol groups, are also potent vasodilators. Our experiments will show whether nitric oxide reactivity is preserved through the formation of nitrosothiols on the erythrocyte membrane. This potential finding could have significant ramifications in the current understanding of erythrocyte structure and function.
- Continuation of the reactivity studies of sulfhydryl groups on the red cell membrane as a function of storage time.

Christensen

- Using a filter apparatus, deformability of stored red blood cells will be evaluated. The "filterability" or deformability of these cells is an important determining factor on the survivability of these cells in vivo. Therefore, the results of these experiments will have important implications on future studies. In addition, the other four in vitro parameters will be measured on a regular basis.

Gabel

- The microstructures will continue to be optimized as an in vitro test for red blood cells. The behavior of specifically altered red blood cells will be further studied, using the microstructures. In particular, cells with modified membrane surfaces will be investigated. Finally, blood that has been stored for extended periods of time (using the advanced storage techniques developed by Dr. Yoshida and Dr. Bitensky)



will be studied using the microstructures. By observing the behavior of the stored blood and comparing it to previous tests, we hope to learn specific information about the red blood cells' physical health.

Gordon

- The primary goal of designing bacteriological assays for microbes found in stored blood will be expanded and refined and detection methods investigated.

Mark

- In the next quarter Julia Mark will continue to study the phosphorylation levels of the red blood cells in the capillaries in order to understand the red blood cell's ability to deform and circulate. Future goals include phosphorylating and dephosphorylating the proteins in the cytoskeleton and running them on the 2-D gels to determine the different patterns between phosphorylated and unphosphorylated proteins. She will also learn how to freeze the red blood cells in a kinase/phosphatase buffer in order to preserve the phosphorylation level of the proteins in the capillaries.

Lee

- Not applicable.

Tarr

- The chemical glaciation of red cells will be tested and all available in vitro diagnostics applied to the products. We will also evaluate the use of glycosyl transferases to terminally decorate existing oligosaccharides on the cell surface. The aim here would be to add one or more of the sialic acid analogues that are known to be resistant to enzymatic removal in vivo. These modifications may help red cells to survive storage and subsequent transfusion.
- Further efforts will be made to render mass spectrometry usable for analysis of the red cell cytoskeleton. As loss and rearrangement of cell membrane are probably responsible for most of the remaining problems with long-term storage, any improvement in the ability to detect these changes is highly desirable.



Thomas

- Continue to provide support to HFPA, Army, Navy and COE staff.
- Continue producing working drawings which can be effectively used for space utilization and management in the new health facility.
- Continue to maintain all CAD workstations.
- Continue to provide support of CMS database.
- Stay abreast of any new CAD features, processes or training which may assist in the design of the working drawings and arrange for any further training as necessary.

Yoshida

- We will design and fabricate a prototype anaerobic blood storage bag with the oxygen sorbent manufacturer, Multisor Technologies. In the past year, we have focused on optimizing ATD, production and hemolysis and developing and OFAS1 storage solution. We are now launching a multi-pronged storage experiment to achieve the best possible compromise in optimizing these parameters (minimal hemolysis, minimal vesicle production, optimal ATP), while at the same time achieving and preserving an ideal cell deformability so that the stored cells are in an optimized state for return to the circulation.



II. NMRI, Bethesda, MD

C. BIOMEDICAL DIVING RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Porter

- To support in the selection and testing of a hyperbaric CO2 analyzer for fleet submarine dry deck shelter use.
- To support analysis of fleet soda lime for possible contamination and to analyze the samples for specific dye concentrations when indicated.
- To assist with other laboratory duties as needed.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Porter

- To continue the testing program for the hyperbaric CO2 analyzers approved for fleet use.
- To began work on new tasking to sample air compressors on fleet submarines.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Porter

- A laboratory testing has been completed for the six initial CO2 analyzers.
- Thirteen additional CO2 analyzers have been ordered for transition into fleet use.
- Six of the new CO2 analyzers are currently undergoing laboratory testing.
- Orders have been placed for material to support new tasking to sample all compressors on fleet submarines. Testing scheduled to begin summer 1997.
- Preformed other laboratory as requested.



GEO CENTERS, INC.

Publications

- Co-authored a scientific paper "Decompression comparison of helium and hydrogen in rats." R.S. Lillo, E.C. Parker, and W.R. Porter, *J. Appl. Physiol.* 82(3): 897-901, 1997.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Porter

- To continue analysis of fleet soda lime for contaminants and dye concentration as needed.
- To continue testing program for dry deck shelter hyperbaric CO2 analyzers that will be issued to Seal Delivery Teams for fleet use.
- To continue work on new tasking to develop and implement a field test plan for divers air bank sampling on 688 class submarines.



II. NMRI, Bethesda, MD

B. PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

McCowin

- Provide management support to the Special Operations Forces Medical Technology Development Program at the Naval Medical Research and Development Command. Duties include reviewing and evaluating medical research proposals, reviewing incremental reports and comparing them with the approved research plans, recommending guidance, and drafting periodic and ad hoc management reports, developing presentation materials and managing financial budget. The scope of research includes all topics within the Special Operations Forces Medical Technology Development Program. This includes investigations relevant to the treatment of disease, trauma, effects of environmental extremes and treatment for medical support of Special Operations Forces Operations. In addition, from time to time, collect, process and report findings on critical issues which are directly related to other urgent military medical research issues within the purview of the Special Operations Forces Medical Technology Development Program.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

McCowin

- Collect monthly obligation and expenditure reports from principal investigators.
- Evaluate and distributed 2nd incremental progress reports for FY97.
- Evaluate and distribute proposal submissions for the FY98 new start project.
- FY96 and FY97 obligations and expenditure report and obligation plan to Special Operation Acquisitions Center (SOAC) for Execution Review Conference.
- Submit monthly FY96 and FY97 unobligated funds report to SOAC.
- Plan and coordinate Jun 97 Program Review.
- Attend Biomedical Initiative Steering Committee (BISC) meeting Jun 97.
- Evaluate and reorganize work unit file of principal investigators for funding and deliverable status.



GEO CENTERS, INC

- Maintain the Program Base Accounting System (PBAS) for maintaining USSOCOM funding.
- Prepared briefing slides for all USSOCOM funded projects.
- Work in conjunction with Booz Allen & Hamilton, Inc. to prepare a USSOCOM Med-Tech Program Briefing Package to be delivered to USSOCOM Component Command Surgeon's.
- Prepare and updated files of all USSOCOM for the incorporation into new 3-D Unix-based computer program system.
- Update Work Unit Information Summary for FY97 submission.
- Prepare transfer of all program management files to USSOCOM.
- Prepare expired and closing account report for OSD.
- Distribute remaining FY97 funds to project managers.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

McCowin

- Work from reporting period objective section (I) was performed during this reporting period.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

McCowin

- Attend Special Operations Medical Association (SOMA) Meeting and USSOCOM BISC Meeting in Dec 97.
- Collect and evaluate 3rd incremental progress reports.
- Collect monthly obligation and expenditure reports from principal investigators.
- Submit monthly obligation and expenditure reports to SOAC.
- Provide input for the reversion of the USSOCOM Project Reference Book.

II. NMRI, Bethesda, MD

E. BREAST CARE CENTER

DESCRIPTION OF WORK TO BE PERFORMED

Patient Service Representatives

Grimes, Jenkins, Kidwell, Williams

- Process and interview patients, incorporate standard patient registration procedures. Maintain uniform policy for check-in/check-out procedures.
- Collect third party insurance forms on each patient.
- Receive patients and incoming telephone calls/inquiries, determine priorities and refer to proper person/department.
- Ensure that all incomplete patient records and third party forms are corrected or returned to proper staff for completion/correction.
- Set up records and filing system for paperwork associated with each patient record. Ensure that all documents processed are in accordance with department standards and that all forms are in designated order in the patient records. Label files for permanent shadow files.
- Orient new support team members and clinical team staff to office routine.
- Call all no-shows, record reason for not keeping appointment in shadow file and initial.
- Print Composite Health Care System (CHCS) daily schedule and end of day reports. Check end of day report for accuracy.
- ADS System: Educate providers, ensure completeness/accuracy of ADS forms, scan forms.
- Inform Technical Assistant of supply levels.

Balintona

- Responsibilities include addressing the psychosocial status, mental status, patient concerns, and the impact of diagnosis on family relationships of breast cancer patients.
- Assess newly diagnosed breast cancer patients and provide them with social work educational materials. The assessment includes a screening for depression, adjustment, patient social history and support systems available to patient.
- Facilitation of the Stage I & Stage II Breast Cancer Survivors= Group



GEO CENTERS, INC.

- Facilitation of the Advanced Breast Cancer Support Group on Thursday mornings at 8:30am
- Facilitate the Spouse's of Breast Cancer Patient's Support Group.
- Collect and analyze research data on the Adjustment and Social Support in Male Spouse's of Breast Cancer Patients.
- Liaison with the National Naval Medical Center Social Work Staff. Attend all social work staff meetings to coordinate communication and colleague interaction.
- Coordinate individual, family, group and marital psychotherapy based on Social Work assessment and clinical intervention needed
- Key contact person for the Look Good, Feel Better program run in the Breast Care Center. This program is offered in our center to facilitate coping and increase patients' self-esteem during radiation and chemotherapy treatments. The responsibilities of this program are to keep monthly contact with the American Cancer Society and promote the program to our patients.

Blankenship

- Provide care for patients with both malignant and benign diseases of the breast, including initial evaluation, definitive medical or surgical therapy, and long term follow-up.
- Educate medical students, residents, nurses, and other physicians in the diagnosis and treatment of malignant and benign diseases of the breast.

Durand

- Acts as a liaison between the patient and the family and all other health care providers, intervening at key points (and or when significant problems occur) for individual patient. Addresses and resolves issues that have a negative impact, creating opportunities and systems to enhance positive outcomes.
- Performs on site visits with patients in various clinical areas.(i.e. Radiology Oncology (Rad. Onc.) Medical Oncology (Med. Onc.) and Post-Op areas.
- Initiates and contributes via multidisciplinary team approach modifications or changes in care giver practice patterns to maximize quality patient care and resource utilization.
- Assists in the development and implementation of the Care Central program with Ellora Corporation.
- Checks daily for outstanding biopsy results.
- Page and inform physician of biopsy results if positive. (when necessary).

- Assists with the pre-operative process of patients, to ensure a smooth transition into the operative phase of their treatment.
- Make follow-up phone calls to post operative patients to check on their well-being, this can be weekly, monthly, and every 3 months for maximum of 6 months, then prn.(whenever necessary.)
- Schedule follow-up appointments to Rad. Onc, Med. Onc, Physical Therapy, Nuclear Medicine, and CT Scan etc. and various other referrals when ordered.
- Verify consults to clinical areas for breast cancer patients with follow-up phone call.
- Follow-up visit to clinical areas with patients after surgery.
- Verify surgical dates via surgical clinic and OR schedules.
- Provide pre-op teaching in conjunction with the physician, and nurse educator for the patients and their families regarding alternative treatments for breast cancer (Required by the Dept. of Health and Mental Hygiene.)
- Distribute educational material for treatment options to patients and their families. clarify any additional information when necessary.
- Acts as support system for patient and family, in conjunction with the Social Worker and Nurse Educator for newly diagnosed cancer patients.
- Instructs post-op mastectomy patients on breast prosthesis, and issues dealing with the appointments for fitting. Then make appropriate referrals to the social worker, who will initiate the order forms with prescription for the acquisition of the prosthesis.
- Acts as liaison between prosthetic company and patient, social worker, and physicians.
- Hand delivers consults to various clinical areas.
- Follow-up with overseas patients, to assure their re-entry into the system when appropriate.
- Verify the transfer of slides/blocks/patient documentation between institutions.

Richman (Fields)

- Perform technical services including mammograms.
- Assisting in biopsies and ultrasounds.

Higgins

- Participate in Hastings Center education program for practitioners
- Organize and schedule the next BRCA Education Group
- Continue to recruit and register patients for participation in Tam/4-HPR



- Conduct individualized patient information sessions for BRCA
- Attend a demonstration of "4th Dimension" software for data collection
- Continue to generate diagnosis/treatment records for QA of breast cancer patients charts
- Patient education/counseling relating to protocols
- Continue to act as liaison between BCC and other governmental/research institutions
- Utilizing Care Manager to identify trends of care in the BCC and to document nursing notes
- Keeping the BCC staff abreast of research issues relevant to patient care and staff development
- Attending seminars/conferences for staff and professional development
- Continue to attend Graduate School to further enhance nursing knowledge
- Participation and case study presentation at BCC staff meetings and multidisciplinary meetings
- Update and maintain protocol log books
- Organizing patient charts for BRCA and obtaining pathology reports

Louie

- Serve as mammographer in the department of radiology at National Naval Medical Center (NNMC).
- Serve as consult for referral cases from outside institutions as well as the Breast Care Center (BCC) here at NNMC. Many of these are complex cases which are sent to NNMC for further evaluation or a second opinion.
- Serve as liaison between the medical staff in the BCC and the mammography section of the radiology department.
- Serve as consultant radiologist for weekly surgical tumor board meetings.
- Supervise the radiology resident assigned to the mammography section of the radiology department.
- Serve as consultant to radiology staff regularly rotating through the mammography section.
- Supervise the mammography technologists to insure that the mammograms meet American College of Radiology (ACR) and Food and Drug Administration, Division of Mammography (FDA) standards for mammography accreditation.
- Investigate, initiate and participate in the planning of other mammography research projects in which NNMC may be a participant.



McIntyre

- Support a research program which focuses on breast cancer.
- Liaison between the Radiology Department-Mammography Section, the Breast Care Center (BCC), and other hospital departments.
- Perform nursing duties.
- Perform managerial duties.

O'Halloran

- Collaborates with a multidisciplinary staff concerning patient needs and identifies patients who may benefit from services such as social service, physical therapy or nurse case management.
- Performs professional nursing assessments
- Opening and closing all clinical areas and preparing exam rooms for patient use
- Triage of telephone calls and patient walk-ins
- Responsible for all clinical functions
- Acts as Relief Clinical Nurse Manager, in the absence of the Nurse Manager
- Carrying out of physician's orders
- Reviewing and sorting of pathology and mammogram reports
- Overseeing preparation of charts for patient visits
- Assignment of nursing lunch breaks to ensure appropriate coverage of the unit
- Processes linen and hazardous material
- Check and order supplies for clinical exam rooms and needle/syringe cart
- Coordinate all FNAs and procedures and notify Nurse Case Manager of positive diagnosis
- Attends seminars/conferences
- Performs biopsy teaching and APU coverage in absence of Clinical Educator

Prindle

- Coordinates patient flow activities
- Collaborates with physicians concerning unscheduled patient appointments
- Performs professional nursing assessments
- Teaches breast self examination and pre and post biopsy education
- Triages patient phone calls and consults with physicians as needed
- Prepares patient charts with appropriate medical, lab, and x-ray reports
- Responsible for entering physician orders into computer



- Assists physicians with all procedures such as FNA or cyst aspirations
- Provides physical and emotional support to patients during their appointment
- Collaborates with a multidisciplinary staff concerning patient needs and identifies patients who may benefit from services such as social service, physical therapy, or nurse case management
- Management of clinical supply needs
- Rotate team leader and triage position

Rapson

- Coordinate patient flow activities
- Perform professional nursing assessments
- Teach breast self examination
- Prepare patient charts with appropriate medical, lab ,and xray reports
- Assist physicians with all procedures such as FNA or cyst aspirations
- Provide physical and emotional support to patients during their appointment
- Collaborate with a multidisciplinary staff concerning patient needs and identifies patients who may benefit from services such as social service, physical therapy, or nurse case management
- Responsible for preparing all clinical areas for patients and securing clinical areas at the end of the day
- Process linen and hazardous wastes within the BCC
- Maintain supplies at par level and records supplies needed

Rogers

- The social worker will interview and assess newly diagnosed breast cancer patients and provide them with educational materials, support group information, and a description of available social work services. The assessment will include a screening for depression and adjustment, the documentation of the patient's social history and a defining of the patient's environmental support systems.
- The social worker will evaluate and monitor the breast cancer patient's psychosocial and mental status and offer individual, couple, family, or group psychotherapeutic intervention or referral as appropriate.
- The worker will facilitate and encourage the identification of the patient's concrete needs and concerns and actively participate with the patient in a solution and task focused pursuit of such.



- The social worker will provide the..facilitation of the Stage I & Stage II Breast Cancer Survivor Group
- Facilitation of the Advanced Breast Cancer Support Group, and facilitation of the Spouses of Breast Cancer Patients Support Group
- Solicit new member participation in the afore mentioned Breast Care Center support groups. Collect and analyze the support group research data related to the Adjustment and Social Support in Male Spouses of Breast Cancer Patients. Act as the liaison between the Breast Care Center and the National Naval Medical Center Social Work Department. As such, the worker will attend all social work staff meetings, offer professional coverage and other services when necessary, coordinate communication, and maintain collegial rapport and interaction. Coordinate Breast Care Center patient participation in the American Cancer Society "Look Good, Feel Better" program for patients undergoing or having completed radiation and chemotherapy treatments.

Snee

- Case manages new breast cancer patients
- Utilizes the "Care Manager" software to document and track the patient' s progress through the clinical care pathway of breast cancer treatment
- Helps to educate newly diagnosed breast cancer patients about disease, treatment, and follow up care
- Provides educational materials to patients and families
- Coordinates and plans appointments for multidisciplinary care in hospital, including, but not limited to hematology/oncology, radiation/oncology, plastic surgery, physical therapy, and social services
- Teaches patients about prosthetics and assists patient in preparing appropriate forms necessary to obtain prosthetic
- Provides emotional support to women and their families who are facing cancer treatment through verbal and nonverbal communication
- Provides support, comfort, and education to the patient through the use of pre and post op phone calls and by visiting the patient while they are an inpatient.
- Ensures that patients are receiving adequate follow up care
- Tracks breast biopsies and notifies doctor of any malignant pathology reports and ensures that patient is scheduled for appointment with physician
- Teaches and demonstrates the "Care Manager" software to interested personnel both within NNMC and at outside facilities
- Assists as needed in clinic as either ambulatory care nurse or nurse educator



Snyder

- Develop and integrate a breast care educational program for female/male Department of Defense beneficiaries and their support persons.
- Educational program to include all breast care issues with an emphasis on early detection of breast cancer.
- Provide pre-operative teaching and educate patients regarding breast cancer and treatment options.
- Being available as an information resource person for the patient and their support person.
- Plan staff development programs and maintain BCC staff development records.
- Act as relief Ambulatory Care Nurse under the direction of the nurse manager.
- BCC designated safety representative, responsible for safety manuals, monthly safety meetings and BCC safety issues.
- BCC representative on the Education Council Committee.
- BCC representative on the Nurse Practice Committee.

Taylor

- Manage and maintain the conference room schedule and database.
- Write, edit, and update correspondence.
- Maintain electronic filing system.
- Orders supplies and maintain the procurement process and database.
- Generate reports relative to supply issues
- Point of contact for procurement and the conference room
- Organize supplies and monitor supplies on hand

Vaughn

- Medical filing for the Radiology department and the Breast Care Center.
- Enter CHCS orders for comparison mammograms.
- Track mammogram films.
- Handle mail and telephone correspondence regarding radiology films.
- Pull and file mammograms.
- Make copies of mammogram films for physicians.

Wallace

- Act as Administrator of the Breast Care Center, responding to the needs of patients and staff to meet daily administrative requirements
- Oversee/Manage appointment scheduling system that allows for: maximum access of patients into the clinic, provides for medical training, research protocols, and administrative time, and is responsive to unanticipated demands and special cases.
- Gather workload data, prepare statistical reports, and analyze data to provide information and guidance.
- Patient ombudsman for the Center during Nurse Manager's absence.
- Coordinate input in order to prepare the annual budget, mid-year reviews, and unprogrammed requirements for the Center. Provide recommendations to the administrative team in the development and formulation of budget requests, based on familiarity and knowledge of Department programs and appropriate procedures, review and analyze budget requests, and determine whether requests for funds and expenditures are proper, necessary, and timely. Monitor use and rate of expenditures of budgeted funds. Oversee funding for all research conducted at the Center, with particular emphasis on clinical trials.
- Responsible for coordinating responses to all correspondence that comes into the Center. This includes Congressional inquiries, complaints, requests for information, requests for guest speakers, etc.
- Coordinate all reports generated in the Center. This includes establishment of a system that will guarantee reports are on time and that all reports reflect accurate data.
- Maintain oversight of equipment inventory and ensure that equipment is maintained.
- Review space utilization within the Center and advise the administrative team on such activities as space allocation and renovation.
- Supervise GEO-CENTERS, INC personnel located in the Breast Care Center, Building 10, 4th Floor, West..
- Manage information systems hardware and software within the Center. Primary coordinator for CHCS within the Center.
- Maintain oversight of the ordering process for supplies.
- Primary liaison between the military and GEO-CENTERS, INC.
- Provide advice on manpower utilization, work flow, and operational procedures.
- Respond to requests for administrative reports; generate, collate, synthesize and present a wide range of data in written or oral form; edit reports prepared by other members of the Department; and, confer with the administrative team in identifying and resolving administrative problems and needs.



- Coordinate staffing with the Nurse Manager, analyze manpower utilization and participate in interviews.
- Monitor legal issues. Make Staff Judge Advocate's office aware of potential litigation.
- Work with administrative team to develop plans for guiding future clinic operations.
- Oversee use of the Ambulatory Data System (ADS) for the Center.
- Assist Contract Management Department with maintaining accurate and complete files on contract employees.
- Assist in preparation for VIP tours and briefings.
- Other administrative functions as necessary.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Patient Service Representatives

Grimes, Jenkins, Kidwell, Williams

- Change patient chart system.
- Modify division of duties based on personnel changes and elimination of rotating positions.
- Streamline and organize front-desk procedures.
- Retrieve and ensure completion of third party insurance forms
- Improve routing and response to incoming telephone calls/inquires
- Use standard registration procedures requiring plastic green card for imprinting all forms pertinent to each patient.
- Coordinate policies for scheduling appointments/procedures for patients calling/walk-ins/consults/cards.
- Streamline physician schedule notification process.
- Refine CHCS daily schedule and end of day reporting.

Balintona

- Turnover position to new Social Worker. Transition out. Transfer patients to new Social Worker.

Blankenship

- Maintain the high standard of care that has been established at the Breast Care Center and provide comprehensive care to patients with diseases of the breast.

Durand

- Continue to improve Care Central through ongoing collaboration with Ellora Corporation.
- To continue to develop through multidisciplinary collaboration, the Nurse Case Manager's position.
- Continue to enter in "Care Central" program all newly diagnosed Breast Cancer Patients/ All new Biopsies, and patient documentation.
- Refine techniques for capturing statistical data that would impact future studies of the Breast Care Center.
- Continue with the ongoing consultation of Ellora Corporation.
- Continue to develop case manager guidelines for the new Breast Cancer diagnosed patients.
- Continue to improve the guidelines for the consult process to the various providers.
- Effectively manage resources in the care of patients.
- Continue ongoing data entry to maintain the statistical information gathered in the BCC.
- Continue to review patient's outcomes, to insure that they are met in a timely, and a cost effective manner.

Richman (Fields)

- Perform various studies within the department thereby increasing knowledge and experience.
- Broaden understanding of the BCC's procedures and personnel. Expand relationship with BCC.
- Take full advantage of any educational opportunities which may arise as time and schedule permits.
- Continued to increase knowledge of mammography and breast diseases using the doctors as teachers.



Higgins

- BRCA pathway/algorithm sheet
- New charts for BRCA patients
- Created BCC pedigree form and key
- Patient referral form for BRCA/Initial contact
- Research protocol binder to be kept in Physician's Library
- Create patient information booklet to provide information post education session
- Develop the slide show presentation for education of BRCA testing
- Utilizing NCI Investigator's Handbook to review research process
- Data collection forms and log-books of patients on protocol
- Attend a demonstration of "4th Dimension" software for data collection
- Continue to discuss and plan data base development within the BCC
- Excel data for BRCA information

Louie

- Continue to follow and further develop the protocols established in the mammography section for evaluating patients with breast abnormalities.
- Continue to perform stereotactic needle core breast biopsies.
- Continue to perform and increase the number of ultrasound guided procedures of the breast, as well as ultrasound scans of the breasts for focal abnormalities.
- Continue to supervise and teach the radiology residents rotating through the mammography section.

McIntyre

- Assist the Radiologists/staff with stereotactic and ultrasound guided breast biopsy procedures.
- Perform assessments on all stereotactic/ultrasound biopsy patients and provide these patients with post breast biopsy teaching instructions.
- Assist with continued development between the BCC and Radiology Department, as the patient volume increases.

O'Halloran

- Organization of patient charts
- Maintain mammography scheduling book

- Modification of daily clinic schedules
- Utilization of Care Manager for tracking of FNAs
- Continue to improve computer skills
- Identify nursing roles for ambulatory care setting
- Development of orientation for new nursing staff
- Stocking of all clinical areas
- Breast self examination teaching
- Organization of triage area and triage files

Prindle

- Continues development in the role of the ambulatory care nurse
- Continues development of computer skills, especially the use of the hospital system called CHCS
- Ongoing evaluation and revision of nursing assessment tool
- Continue to gain further knowledge and education in breast cancer and its treatment
- Ongoing development and assessment of nursing protocols for telephone triage
- Continue working with Patients Service Representatives to achieve a fluid transition between PSR/Patient/Nurse (Developing Algorithm format)
- Train new staff nurses in clinic procedures
- Analyze need for new appointment template system to better utilize patient flow
- Develop new algorithm format with Digital for clinical process
- Increase nursing continuing education instruction

Rapson

- Continue development in the role of the ambulatory care nurse
- Continue development of computer skills, especially the use of hospital's system called CHCS
- Continues to gain further knowledge and education in breast cancer and its treatment

Rogers

- Address the psychosocial and concrete needs of individual patients in the Breast Care Center.
- Provide individual psychotherapy to patients experiencing significant emotional distress following diagnosis.



- Facilitate on-going therapy for patients who have experienced specific types of concerns at the completion of treatment including sexuality and intimacy issues, fear of recurrence, family concerns, etc...
- Facilitate psychotherapeutic intervention with couples who wish to enhance coping skills and increase the level of communication, sense of well-being, and stability in their union during a time of dramatic change and crisis following the diagnosis of breast cancer.
- Work toward beginning the American Cancer Society "I CAN COPE" program at the Breast Care Center. This program will serve to help breast cancer patients to communicate and network with other survivors throughout diagnosis, treatment, and beyond.
- Develop social work involvement with the BRCA Gene Study. Social Worker will serve as individual providing therapy to patients who experience anxiety, depression or other feelings related to the gene testing process and results of the tests. Examining the option of starting a genetic support group.

Snee

- Ongoing development in the role of the nurse case manager
- Implement processes that will enable appropriate follow up care for breast cancer patients
- Continues to revise and perfect methods to discuss cancer diagnosis with patients
- Continues to gain further knowledge and education in breast cancer and its treatment
- Ongoing development of organizational skills to manage multiple patients and their individual needs
- Continues to provide "Care Manager" demonstrations to interested parties coming to the BCC

Snyder

- Continue in the learning role of the Clinical Educator.
- Continue to provide patient education.
- Continue to develop array of patient educational materials.
- Continue to act as relief ambulatory care nurse.
- Continue staff development and safety representative responsibilities.



Taylor

- Streamline and organize office procedures to promote a work smarter environment.

Vaughn

- Alphabetize the main mammography file system.
- Systematic checking for quality improvement.
- Improve report filing to allow for more efficient operations.
- Being readily available for assistance to co-workers, the BCC staff, physicians and patients requiring assistance with mammography films.

Wallace

- Oversee conversion of patient charting system. Ensure appropriateness of conversion plan. Develop timeline for conversion.
- Become familiar with position as Administrator.
- Analyze current staffing and future staffing needs. Determine effectiveness of current nursing staff positions. Hire personnel as necessary. Orient new employees.
- Monitor Breast Cancer Prevention, Education, Diagnosis Initiative issues closely. This includes preparation of Statements of Work and proposals for obligation of future funding and completion/ submission of expenditure of funds reports to the Office of the Assistant Secretary of Defense and Tricare Region 1 Lead Agent's office.
- Further investigation of the integrity of data, with particular attention to use of CareCentral Software.
- Continue to maintain compliance with the Surgeon General's ADS standards.
- Attend weekly meetings of the Information Management Quality Management Board to keep up-to-date on all information systems issues.
- Oversee procurement ordering process. Make sure all necessary supplies are ordered in a timely fashion. Ensure proper documentation.
- Participate in genetics research and cancer database development working groups.



SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Patient Service Representatives

Grimes, Jenkins, Kidwell, Williams

- Divided duties among remaining 3 Patient Service Representative Positions. Trained temporaries.
- Continued organization of front-desk procedures
- Assisted in development of standard operating procedures.
- Processed and interviewed patients through CHCS and designated forms, obtained and updated all patient demographic information and ensured completion of forms.
- Obtained and verified pertinent insurance information utilizing available forms. Obtained third party insurance forms from physicians at end of each visit.
- Required identification card from each patient and imprinted all clinic forms pertinent to that patient.
- Received patients and incoming telephone calls/inquiries, determined priorities and referred to the proper source.
- Explained clinic procedures to patients.
- Retrieved/returned Mammogram films daily.
- Obtain authorization for release of mammogram films from patient, for NNMC file tracking.
- Open monthly clinic schedules and make changes as necessary, based on physician schedule changes.
- Ensured completion of incomplete patient records and third party insurance forms.
- Set up records and maintain filing system for paperwork associated with each patient record. Ensured that all documents processed are in accordance with department standards. Filed all forms in designated order in patient record. Labeled files for permanent shadow files.
- Scheduled and coordinated front desk procedures in accordance with department policy. Identified process problems and helped develop suitable solutions.
- Oriented new support team members and clinical team staff to office routine.
- Participated in team planning to assure team members meet team quality standards. Maintain department standards of productivity.
- Notified physicians the day before they are scheduled for clinic; let them know approximately how many patients they will have.
- Continue working with the Ambulatory Data System (ADS):



Balintona

- Addressed the psychosocial status, mental status and patient/family concerns in the Breast Care Center
- Worked closely with the CHAMPUS office to ensure that patient's breast prosthetics and wig paperwork is approved by CHAMPUS prior to patient's purchase of these items.
- Compiled a list of wig salons for patient who are undergoing chemotherapy. This further enhances patient access to services and empowers patient to locate a wig which can increase self-esteem.
- Development of the Social Work Assessment that can be completed on computer. This enhances the social workers efficiency because of time savings and rapid chart documentation.
- Worked closely with the BCC Nurse Case Managers to provide seamless care to patients. This includes daily integration and discussion of services provided to ensure patient care continuity and enhanced patient satisfaction.
- Liaison with the National Naval Medical Center Social Work Department. This included integration with the Social Work department with the signing of a Memo of Understanding signed by the director of Breast Care Center and NNMC Social Work Department during the current reporting period. This ensures that social work coverage will be provided in absence of BCC social worker (during a time of unplanned illness or planned vacation days).
- Completed Turnover

Blankenship

- Provide comprehensive care to patients with diseases of the breast.
- Provide feedback on patient care processes and suggest improvements.

Durand

- Have assisted Ellora Corporation in the ongoing development of the "Care Central".
- Have successfully entered approximately 15 new Breast Cancer patients, and 37 new diagnostic procedures in the Care Central system.
- Have acted as liaison between patient and providers in their treatment plan.
- Have generated statical data for monthly evaluation within the Breast Center.
- Have participated in the education of Nurse Case managers, and other health care professionals in the "Educate the Educator" program in the Breast Center.



- Have been consistently available for consults for patients, and their family members when necessary.
- Have assisted in the Nursing research project, for various protocols by making patients aware of the program.

Richman (Fields)

- Performed a variety of mammograms, stereotactic biopsies, needle localizations and ultrasound procedures.
- Interfaced with mammography doctors to increase knowledge in the areas of mammography and breast disease.
- Became more familiar with the BCC personnel.
- Continued follow-up for screening mammogram program.
- Attended lecture on "Stereotactic Biopsy" with Dr. Claudia Galbo.
- Attended lecture on "Epidemiology of Breast Cancer" with Dr. Miguel Kamat.
- Attended 2 day mammography training course at NNMC with DOD.

Higgins

- Updated Tam/4-HPR pamphlets
- Created Tri-fold brochure for BRCA
- Developed nursing in-service program for staff education related to BRCA
- Created web-site statement for Tricare to advertise BCC protocols
- Recruitment of patients for participation in research
- Conducted individualized and group patient information sessions for BRCA
- In-service to APU/SSU staff
- Hastings Center teleconference
- Panel member for BCC nursing interviews
- Patient education/counseling relating to protocols
- Liaison between BCC and other governmental/research institutions
- Utilizing Care Manager to identify trends of care in the BCC and document nursing notes
- Keeping the BCC staff abreast of research issues relevant to patient care and staff development
- Attended seminars/conferences for staff and professional development
- Attend Graduate School to further enhance nursing knowledge
- Case study presentation at BCC staff meetings and multidisciplinary meetings
- BRCA patient pathway



- Nurse Case Manager Curriculum presentations
- Collaborated with nursing staff on the re-definition of roles within the BCC
- Completed BRCA slide show presentation

Louie

- Continue to serve as one of the two principal mammographers in the department. I am more frequently on the schedule and read more mammographic studies than any other radiologist assigned to the section. There are 1 to 3 half days each week when I am the only mammographer assigned to read films.
- Continued to perform stereotactic needle core biopsies of the breast as well as needle localizations for surgical excisions, on a regular basis.
- Drafted a proposal to demonstrate the feasibility of imaging the breast with fluorodeoxyglucose (FDG) using a gamma camera. This procedure has never been published before, and will involve a collaboration of the nuclear medicine and mammography departments of NNMC. The proposal will be edited and then submitted to the Investigational Review Board (IRB).
- Increased involvement in teaching the radiology residents as they rotate through the mammography section.
- Continue to identify interesting cases to add to the resident teaching file.
- Begin to identify very high risk patients who may be interested in the BRCA gene education and screening program now offered by BCC. These names are forwarded to BCC for future contact.
- Arranged for a dedicated transcriptionist for mammography. This has resulted in more rapid transcription of reports with significantly fewer transcriptional errors.
- Participate in the regularly scheduled BCC research meetings as to ongoing and potential projects involving BCC.
- Preliminary talks with investigators at the National Cancer Institute (NCI) and the National Institutes of Health (NIH) for possible future collaborations.

McIntyre

- The above technical objectives were met during the current reporting period.
- Assisted with the re-organization of the mammography scheduling process.
- Supervised other mammography personnel.
- Completed statistics for FDA/ACR mammography inspection for 6/96-5/97 period.
- Tracked 6 month follow-up patients with outcome analysis via BCC Task Management Tool.



- Assisted with and completed the BCC "Nurse Case Manager (NCM) curriculum" for 7/97- 9/97 period.
- Assisted the BCC with "Educate the Educator" program in the Radiology Department- Mammography section.
- Correlated mammography and pathology findings via CHCS.

O'Halloran

- Participated in multidisciplinary meetings to further enhance the relationship between BCC, SSU and GSC
- Enhance nursing knowledge base on breast cancer issues
- Further developed personal computer skills
- Triage telephone calls and walk-ins
- Further identified the nursing assignments of the ambulatory care staff
- Coordinated all FNAs and procedures and notify Nurse Case Manager if positive
- Continued to orient new staff members to patient flow processes and forms within the BCC.
- Coordinated patient flow activities in the clinical areas with patients, nurses and physicians

Prindle

- Coordinated patient flow activities
- Collaborated with physicians concerning unscheduled patient visits
- Performed professional nursing assessments
- Provided BSE and biopsy teaching
- Triaged patient phone calls and made telephone consults to physicians
- Prepared patient charts appropriately with medical, lab, and x-ray reports
- Entered physician orders into the computer
- Assisted physicians with procedures done in the BCC
- Provided physical and emotional support to patients
- Collaborated with social service, nurse case manager, clinical nurse educator, physical therapist and many physicians to ensure exceptional patient care
- Assisted the Reservist RN in her role for "Educate the Educator Program"
- Developed and designed a BSE poster for outside NNMC clinics with the Reservist RN

Rapson

- Coordinated patient flow activities
- Performed nursing assessments
- Provided BSE teaching
- Prepared patient charts appropriately with medical, lab, and x-ray reports
- Assisted physicians with many procedures done in the BCC
- Provided physical and emotional support to patients
- Collaborated with social service, nurse case manager, clinical nurse educator, physical therapist and many physicians to ensure exceptional patient care
- Disposed of linens and hazardous wastes appropriately

Rogers

- Addressed the psychosocial status and patient/family concerns in the Breast Care Center.
- Worked closely with the Breast Care Center Nurse Case Managers to provide seamless care to patients. This included daily integration and discussion of services provided to ensure a continuity patient care and enhanced patient satisfaction.
- Provided facilitation of the Advanced Breast Cancer Support Group, the Stage I & Stage II Breast Cancer Survivor Group, and the Spouses of Breast Cancer Patients Support Group.
- Worked closely with the CHAMPUS and Supplemental Care offices to ensure that patients wig and breast prosthetics requirements were approved prior to the purchase of such items.
- Actively promoted the Breast Care Center Breast Cancer Survivor groups resulting in increased new membership.
- Attended all National Naval Medical Center Social Work Department meetings, offered professional coverage, coordinated communication, and maintained constant collegial rapport and interaction.
- Coordinated Breast Care Center patient participation in the American Cancer Society "Look Good, Feel Better" program.
- Compiled a comprehensive listing of wig salons for patients who are undergoing chemotherapy and may need to locate a wig prosthetic as a result of hair loss.
- Developed a comprehensive listing of local lodging with current prices and military or patient discount information for use by patients and their companions.
- Completed preliminary study and consideration of the American Cancer Society "I CAN COPE" program for possible implementation at the Breast Care Center.



Snee

- Participated in the Nurse Case Management Curriculum. For each of the three groups of attendants, I presented my lecture entitled "The Art of Case Management for the Breast Cancer Patient", demonstrated the role of the case manager through role playing with the staff, and talked with each group at my work station at length to demonstrate the software program and to discuss the fine points of case management.
- Assisted in the program design for the Nurse Case Management Curriculum for visiting nurses coming to the clinic to learn case management
- Presented my case management lecture to NNMC nurses interested in case management on July 9, 1997.
- Suggested and implemented useful changes in the care manager software
- Helped to educate patients and families on breast cancer
- Provided emotional support to women from diagnosis to completion of breast cancer treatment
- Collaborated with multiple disciplines to arrange for patient care
- Developed useful methods for managing many varied and complex patients
- Taught many new cancer patients about breast and wig prosthetics and assisted them in obtaining the prosthetics
- Attended tumor board meetings and was prepared to give additional information concerning breast cancer patients if required or requested by physicians
- Collaborated with staff on the development of a cancer database
- Provided education and working demonstration of the "Care Manager" software to interested personnel both within NNMC and to outside facilities
- Collaborated with the nursing staff to begin the redesign of the ambulatory nursing role

Snyder

- Continued responsibility as the designated safety representative of the BCC.
- Participated in command sponsored health fairs.
- Maintained credentialing data base on all Geo-Center employees.
- Plans and institutes staff education calendar and events.
- Functions as Clinical Educator providing teaching on breast self examination, pre and post operative instruction and breast cancer.



- Functioned as relief ambulatory care nurse providing breast self exam teaching, assisting the physicians with physical exams, procedures, and scheduling of diagnostic test when needed.
- Participated in health fair/wellness program.
- Continued to review educational materials and order needed materials.
- Participation and preparation of the Educate the Educator program.
- Preparation and participation of the Nurse Case Manager program.
- Participation on the Educational Council Committee.
- Participation on the Nurse Practice Committee.

Taylor

- Performed word processing for the center's staff.
- Ensured a smooth correspondence flow/distribution.
- Maintained procurement database.
- Switched conference room databases to Schedule +.
- Maintain the electronic filing system.
- Wrote patient correspondence for appointment and medical issues.
- Generated supply reports.
- Answered end user questions.

Vaughn

- Provided assistance to staff requesting help with mammography films.
- Continued to organize log book to improve film tracking.
- Continued to disseminate films to patients via CHCS computer.
- Assisted radiologists with research projects by providing mammogram films.
- Performed increased duties as patient volume increased within the BCC.

Wallace

- Coordinated administrative activities of the BCC. Assumed responsibilities of Administrator.
- Finalized plan for chart conversion; Planned for change to new system.
- Researched reliability of Care Manager data. Continue to determine reasons for inconsistency and develop solutions
- Managed schedule templates, discussed need to change templates with ambulatory care nurses.



- Maintained relationship with Contract Management Department.
- Participated in interviews for Nursing Staff and Patient Service Representative.
- Coordinated with Budget Department to match records, worked with technical assistant to develop formal mechanism for ensuring accuracy.
- Coordinate funding/administrative and contractual aspects of the "Educate the Educator" and Nurse Case Manager training programs.
- Attended GEO-CENTERS, INC. training class "Do You Hear What I Hear"
- Handled administrative issues as necessary.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Patient Service Representatives

Grimes, Jenkins, Kidwell, Williams

- Complete patient chart conversion process.
- Become more proficient in the use of ADS.
- Given the continuing environment of change and the influx of new employees, we will take this opportunity to scrutinize current processes and increase the efficiency of the front desk area.
- Maintain department standards.
- Rotate on a monthly basis as Team Leader

Balintona

- Resignation effective 27 June 1997

Blankenship

- Continue to provide care for patients with both benign and malignant diseases of the breast at the Breast Care Center. Increase the number of patients seen in the Breast Care Center.
- Improve patient charting process, data collection mechanisms, and other patient care processes
- Return to Active Duty 01 September 1997



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Durand

- Continue to work in collaboration with Ellora to improve the "Care Central program"
- Continue to develop the Standards of Procedures for the processes of the Nurse Case Manager.
- For a more effective and efficient process, continue to evaluate the patient outcomes and make improvements, with the collaboration of the multidisciplinary team.
- Continue to be the Nurse advocate for patients and their families.

Richman (Fields)

- Prepare to take mammography certification exam in October 16, 1997.
- Attend an educational mammography seminar.
- Broaden my knowledge of breast diseases and mammography.

Higgins

- Develop one-sheet format for patient satisfaction survey
- Produce final report of BCC first 100 cancer diagnosis
- Register patients on TAM/4-HPR protocol
- Continue to improve the screening process for patient participation in BCC research
- Continue to keep abreast on breast cancer issues using NCI Current Clips
- Continue to further develop personal computer skills
- Continue to attend seminars/conferences on breast cancer issues and professional nursing issues
- Continue to coordinate research proposal/grant development
- Complete Tri-fold and booklet for BRCA
- Contact tumor registry regarding patient identification for BRCA
- BCC chart review to identify high risk and strong family history

Louie

- Continue to provide coverage in the mammography section of the radiology department.
- Submit above-mentioned proposal to the IRB.
- Funding for digital acquisition mammography equipment has been approved for Capt. Jerry Thomas, Uniformed Services University of the Health Sciences (USUHS). He is



interested in installing it here at NNM. Projects will have to be designed for digital acquisition mammography.

- Meet with the members of the Transfer of Intelligence Technologies to Improve Breast Cancer Imaging Project (TITIBCI) regarding preliminary data now being collected.
- Expect to participate as a mammography reader in one, possibly two, digital mammography projects presently ongoing.

McIntyre

- Continue to perform nursing and managerial duties, as described above.
- Continue to obtain mammography statistical data for FDA purposes on a monthly basis.
- Track 6 month follow-up patients with outcome analysis via BCC Task Management Tool.
- Attend nursing/management conferences when available.

O'Halloran

- Continue to enhance nursing knowledge base on breast cancer issue
- Continue to further develop personal computer skills
- Attend seminars/conferences on breast cancer issues and professional nursing issues
- Streamline patient flow processes in the clinical area
- Continue graduate studies to obtain Masters of Science in Nursing
- Development of position description and orientation package
- Continue to re-organize ambulatory nursing structure to support increased patient numbers
- Work towards integration of physicians, nurses and PSRs to improve and support the clinic
- Become more familiar with administrative functions and their affect on nursing in the clinic
- Participation on panel interviews of future nursing staff
- Continue to cross-train in research and nurse case management
- Develop new appointment template system to better utilize patient flow
- Develop new chart system to accommodate physician requests
- Develop new position descriptions of the ambulatory nursing staff



Prindle

- Resigned 08 August 1997

Rapson

- Will continue to enhance education in breast cancer and its treatment
- Will continue to improve patient flow management
- Will continue to improve computer skills
- Will attend a seminar/conference related to breast cancer
- Will continue to participate in multidisciplinary meetings
- Will continue to improve chart review

Rogers

- Continually provide comprehensive and high quality psychosocial and concrete services and interventions to Breast Care Center patients and their families.
- Coordinate and facilitate individual, couple, family and group psychotherapy for breast care patients.
- Continue to prepare and complete CHAMPUS and Supplemental Care documents for the procurement of necessary concrete items by breast care patients.
- Expand the BCC social work library to include more texts that address the emotional issues related to breast cancer.
- Continue working relationship with the American Cancer Society to bring to the Breast Care Center quality programs which address psychosocial issues related to cancer.
- Participate actively in the Educate the Educator Program sponsored by the Breast Care Center.
- Become an active member of the Medical Ethics Committee of the National Naval Medical Center.
- Begin to develop a guided imagery meditation, stress reduction, and relaxation tape for use by breast care patients, their family members and loved ones.



Snee

- Will work to establish guidelines for information entry in the "Care Manager" software
- Will assist the ambulatory care staff with daily activities in the unit as the nursing roles are amended and staff is hired
- Will assist the nursing team to redesign the nursing roles in the clinic, in particular, the roles of the ambulatory care nurses
- Will continue to improve skills as a nurse case manager
- Will assist in the development of a breast cancer database
- Will develop concise methods to manage multiple patients
- Will continue to enhance my education in breast cancer and its treatment
- Will continue to improve computer skills
- Will attend a seminar/conference related to breast cancer
- Will continue to participate in multidisciplinary meetings
- Will establish guidelines for case management follow up after the acute stage of diagnosis and treatment of the breast cancer patient
- Will plan and develop, with nursing and medical personal, a form to be placed in the patient's chart that indicates that patient's individual recommended clinic follow-up schedule after she is diagnosed with breast cancer

Snyder

- Continue responsibility as safety representative.
- Continue to function as Clinical Nurse Educator providing teaching to patients and their support persons.
- Continue to function as relief Ambulatory Care Nurse.
- Identify needed materials and supplies for procurement.
- Participate in Wellness programs.
- Continue participation in the Educate the Educator program.
- Continue staff education calendar and events.
- Continue with the learning role of the Clinical Educator.
- Discussion with a BCC physician regarding preparation of an abstract about the Nurse Case Manager Curriculum.
- Participation in providing education materials for outside staff in October. (Breast Care Awareness month)
- Continue to participate on the Education Council Committee.
- Continue to participate on the Nurse Practice Committee.



Taylor

- Resigned 29 August 1997

Vaughn

- Continue with duties as described above.
- Reduce turn around time for mammogram films returned to the department from the BCC, General Surgery Clinic and patients.
- Purge duplicate mammogram folders.

Wallace

- Orient New Nurse Manager
- Resolve staffing issues. Train replacement for Technical Assistant.
- Increase workload collection. Analyze new areas for data collection. Monitor physician participation.
- Increase knowledge of Tricare. Prepare the Breast Care Center staff for Tricare.
- Prepare for JACHO and Inspector General's visit
- Continue participation on genetics research and cancer database working groups.
- Continue to coordinate administrative activities of the BCC. While developing in position as Administrator.
- Increase budget management, actively seek new avenues of funding.
- Monitor legal issues.
- Monitor compliance between BCC records and Budget Department records.
- Monitor procurement process more closely.
- Actively seek methods to improve current work practices.



II. NMRI, Bethesda, MD

F. DIRECTED ENERGY EFFECTS RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Elliott

- Design and implement a training program for Rhesus monkeys with ultimate goal of animals trained to perform visual acuity tasks while aligned on and being imaged by a Scanning Laser Ophthalmoscope.
- Select and screen candidates for above.
- Install, operate, and maintain Rodenstock Scanning Laser Ophthalmoscope(SLO).

Guillory

- Compile and analyze data comparison of tactical in-flight information versus visual data collected via manual video tracking (spatial analysis).
- Support data collection at remote field sites for future analysis.
- Assist in any logistical support necessary.

Richardson

- Provide Biological Science Laboratory Technician (Animal) Support to the Microwave Department. Handling and training of non-human primates. Recording and compiling data. In-house management of non-human primates. Administrative support of animal use projects.

Thompson

- To provide technical and analytical support for pulsed laser glare projects.
- To provide support in the experimental design and analytical support for visual psychophysical studies.



TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Elliott

- Define Animal Performance criteria and Design Training program: The objective of this research is to evaluate the effects of Q-switched laser exposure on the visual performance and retinal morphology on Rhesus monkeys performing visual tasks while simultaneous retinal imaging with SLO takes place. The preliminary steps of this training program involve a sequence of interim training objectives.
- Selecting Subjects: Suitable Rhesus monkeys must be selected from pool of available animal at BAFB. Candidates must be examined to determine general health, compatibility with training programs, and ophthalmological fitness.
- SLO Installation: Laboratory space must be located, identified, and coordinated with coinvestigators for the SLO and the delivery and installation must be supervised.

Guillory

- Reinforce information to pilots of the threat that hand held lasers pose to any flight operation. Create empirical parameters denoting laser hazard zones allowing the pilot tactical planning before a mission.

Richarson

- At this time will be giving technical support to other technicians with their projects, and assist where needed in animal research. Recording data and compiling data on computer programs for publishing.

Thompson

- To coordinate support for experimental data collection and analysis in the pulsed laser glare project.



SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Elliott

- Training Program Design: A training protocol has been designed, equipment acquired and modified, and the initial stages of animal training begun with excellent progress.
- Subject Screening and Acquisition: Two of the Rhesus monkeys that were screened were found acceptable. They have been placed on the protocol and are in the initial stages of training.
- SLO Installation: The new Rodenstock SLO has been delivered and in routine operation.

Guillory

- Acquired spatial positioning data from actual flight sensor data comparison (data scoring).
- Provided logistical support during data acquisition mission to NSAWC, NAS Fallon, NV.
- Provided daily research and administrative support.
- Attended Laser Safety Class.

Richardson

- Tasked to rewrite the below animal protocols:
 - Proper handling of rhesus monkeys using the Pole and Collar method.
 - Required clothing.
 - Cleanliness of animal environment.
 - Disposal/Disinfection of Contaminated Material and Equipment..
 - Capturing an escaped monkey within the facility.
 - Procedure in the event of escaped monkey outdoors.

Thompson

- Provided scheduling and administered psychophysical testing for laser glare project.
- Maintained subject documentation and organized data collection procedures.
- Provided consultation in purchasing of electronic equipment for future glare study.

- Provided consultation in purchasing back projection screens for future glare study.
- Provided statistical analysis and interpretation of results for glare project.
- Provided consultation for development of psychostimulus for Tri-Service laser project.
- Continued to provide support in the development of the HUD and MFD psychostimulus displays.
- Completed Laser Safety Training course.

GOALS/OBJECTIVE FOR NEXT REPORTING PERIOD

Elliott

- Continue training subjects.
- Prepare new protocol for Animal Use Committee.
- Prepare SLO for table mounting.
- ARVO Presentation May 14: Scanning Laser Measurements in Dorzolamide and Placebo Treated Eyes: Arteriovenous passage times in Large and Small Vessels During Hyper- and Hypo-Capnia. W. R. Elliott*, D. L. Shipman**, J. T. Kavanagh**, W. E. Sponsel**, J. Ness*** (* Geo Centers Inc., Naval Medical Research Inst. Det., Laser Dept., Brooks AFB TX, **Dept. of Ophthalmology, University of Texas Health Science Center, San Antonio TX, ***US Army Medical Research Det. (WRAIR), Brooks AFB TX)

Guillory

- Continue data scoring and logistics assistance.
- Learn more about laser technology and the impact on aviators.

Richardson

- Continued member of the Animal Use Committee. Complete data collection for Publishing. Continue supporting other technicians with various projects, hoping to be assigned a project of my own.



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Thompson

- Completed data collection and statistical analysis of laser glare project.
- Began development and setup of second laser glare laboratory.
- Completed Laser Safety Training course.
- Complete development of second laser laboratory.
- To continue development of the experimental workstation and design a method for simulating aircraft cockpit instrumentation symbology.
- To develop experimental methodology for Phase III of laser glare project.
- To continue to provide statistical support for all laser department projects.



GEO CENTERS, INC.

III. NDRI, Great Lakes, IL and NDRI Detachment, Bethesda, MD

A. DENTAL DISEASES-RELATED RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Beck

- Provide technical assistance with ongoing research projects. Participate in linkage analysis projects with National Institute of Dental Research (NIDR), department of Molecular Epidemiology. Maintain and upgrade the laboratory such that the research experiments are carried out smoothly. Maintain and record proper technical procedures and data produced for each experiment.

Jones

- Senior Research Scientist. Responsible for the Molecular Biological and Molecular Genetic aspects of the projects. This includes the development, evaluation and refinement of molecular biological research protocols.

Miller

- Senior Research Scientist and Group Supervisor. Responsible for all aspects of Immunological, Microbiological, and Tumor Biomarker activities within the Naval Dental School. This includes the development and supervision of research protocols, dental resident mentoring activities, instruction of courses in dental microbiology and dental immunology, serving as a link between NIH sponsored research and Naval Dental Research programs, and troubleshooting of research programs, computers, instrumentation and equipment.



TECHNICAL OBJECTIVE FOR THIS REPORTING PERIOD

Beck

- Assist NIDR with linkage analysis studies of genetic disorders.
- Continue optimizing the polymerase chain reaction (PCR) conditions for cytokine primers such that common PCR conditions can be applied to all sample types, i.e. cell lines.

Jones

- Relative to the program entitled "Biomarkers for Oral cancer" for the Puerto Rico study subgroup, it is anticipated that SSCP and DNA sequence analysis of several of the exons of the p53 gene will have been completed thus allowing the establishment of a mutational profile of this important gene.
- DNAs from the Taiwan Nasopharyngeal Carcinoma Study subgroup will be further characterized using additional genetic markers. The arrival of additional DNA samples for this study is anticipated and these will be incorporated into the ongoing study.
- Analysis of the DNAs from the Taiwan Oral Cancer Case - Control Study subgroup will be expanded with the characterization of additional genetic markers that are associated with increased cancer risk.
- Anticipate clearance for the initiation of work on the Greece Study subgroup samples. DNAs are to be extracted and characterized for polymorphisms within a collection of "high risk" genes. In addition, DNAs derived from tumor samples may be characterized for alterations within specific regions of the p53 gene.
- Anticipate the arrival of additional specimens from the various sites participating in the VA subgroup of the Biomarkers for Oral cancer study. Will begin the extraction of DNAs from these materials and the analysis of genetic variation.
- Anticipate greater involvement in the NNDC Resident projects evaluating the role of bone morphogenetic protein (BMP) receptors in bone regeneration in periodontal tissues.

Miller

- A large number of bone morphogenetic proteins (BMP) and their receptors (BMP Receptors) have been identified in part based on their ability to stimulate bone formation in ectopic extra-skeletal sites. Recombinant BMP has been shown to be



able to induce oral bone *in vivo*. BMP appears to interact with cell surface receptors in a complex manner to stimulate specific actions within cells. BMP receptors have been found in several human and animal tissue cell types; however, no study has identified the presence of BMP receptors in specific oral tissues. A better understanding of the role of bone morphogenetic protein receptors in periodontal and endodontal tissues may help to optimize potential use of recombinant human BMP in regenerative periodontal therapy. These studies will utilize highly sensitive and quantitative reverse transcription polymerase chain reaction techniques in conjunction with ABI 373 Gene Sequencers to evaluate expression of receptor message. This new area of study initially focuses on two projects:

1. Characterization of bone morphogenetic protein receptors in periodontal tissues.
2. Characterization of bone morphogenetic protein receptors in periradicular pathoses.

During the next quarter it is anticipated that a proposal for this work will have been submitted for IRB approval by of the National Naval Medical Center. Primers for use in the PCR procedures have been identified and primer synthesis has begun.

- Relative to studies designed to evaluate antibacterial activity of currently used endodontal medicaments, Vitapex® and Calasept®, this project has been completed and a final manuscript "Antibacterial effectiveness of temporary endodontic filling materials" is nearing completion. It is anticipated that this paper will be submitted to the Journal of Endodontics during the next quarter. This action will complete this program.
- Relative to the program entitled "Biomarkers for Oral Cancer," It is expected that evaluation of the presence of HPV L1, E6, and specific HPV 16 and 18 genes will be completed on Puerto Rico DNA samples. In addition, embedding of samples from the Greece study sub-group will also be done.
- Relative to the project "Changes in Immunoglobulins as a Result of Smoking Cessation and Relation to Neurotransmitter Genes" additional serum and blood samples will be received during the next quarter. Evaluation of levels of IgG2 in these samples will be begun using a radialimmunodiffusion procedure.
- Relative to the project concerning the evaluation of cytokine production by oral fibroblasts, we will continue to optimize the PCR methodology in order to be able to simultaneously run actin and interleukin PCR products on the same gel. In addition, work will be completed on analysis of stimulated endothelial cells.
- Relative to the project "Protein Pattern Recognition for Risk Assessment of Periodontitis" work unit number 0601152N 00004.001.0701 ; we have identified a



summer student to help move this project along. It is anticipated that samples will be evaluated by 2D electrophoresis and analyzed.

Soft denture liners or conditioners are valuable therapeutic materials for short-term reduction of chronic soreness in denture wearers. However, tissue hypersensitivity and cytotoxicity have been reported for such materials. During the next quarter we will begin work on a project entitled "In vitro analysis of cytokine production by fibroblasts exposed to denture soft lining tissue conditioners" involving the effects of Viscogel (Dentsply), Coe Comfort (GC), FITT (Kerr), Lynam (Caulk/Dentsply), and Coe Soft (Coe Lab) on fibroblast growth, cytokine production, as well as determine antibacterial effects on facultative cariogenic bacteria. We expect to submit our proposal for IRB approval during the next quarter.

- Relative to the program entitled "Evaluation of Disproportionate Expression of T-cell Receptor V β regions in Lymphocytes from Patients with Advanced Periodontitis" (Work Unit: 0601152N.MR00001.001-0063, all work has been completed. It is observed that although bacteria associated with periodontal disease etiology carry superantigens disproportionate expression of TCR V β mRNA is not seen in peripheral blood lymphocytes when normal and diseased subjects are compared. A final manuscript is nearing completion and will be submitted to the Journal of Periodontology during the next quarter.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Beck

- Continued to participate in NIDR Molecular and Epidemiology experiments. These studies deal with the inherited genetic disorders. Hundreds of DNA's are gathered from various sites and organized for the genetic analysis. Techniques of PCR and gel electrophoresis are used to investigate gene(s), candidate regions, responsible for the genetic disorder (Ex. cleft lip) being studied. In addition to candidate regions, the entire human genome region is being scanned for a potential marker linkage. This is an ongoing project.



- Significant progress has been made to optimize the primers and PCR conditions for measuring cytokine activities at the nuclear level of various fibroblast cell lines. It will require more fine tuning of protocol before the similar procedure can be applied to all cell lines.

Jones

- Relative to the program entitled "Biomarkers for Oral cancer" for the Puerto Rico study subgroup, "first pass" SSCP analysis of exons 5, 6 and 8 of the p53 gene has been completed. Numerous potential mutants have been isolated and are awaiting characterization *via* DNA sequence analysis.
- Relative to the program entitled "Biomarkers for Oral Cancer," the first of several shipments of the DNAs for the Taiwan Nasopharyngeal Carcinoma Study continue to be characterized for polymorphisms in a large set of genetic markers associated with increased cancer risk. Genotyping studies on these samples is nearing completion. Two additional shipments of DNAs have been received and will be incorporated into the study.
- Relative to the program entitled "Biomarkers for Oral Cancer," clearance for the initiation of work on the Greece Study subgroup samples was obtained. The first set of case/control samples from the study was processed for DNA. Characterization of these DNAs for polymorphisms within a set genetic markers associated with increased cancer risk has been initiated.
- Relative to the program entitled "Biomarkers for Oral Cancer", samples continue to arrive for the VA Oral Cancer Study subgroup and are presently being archived.
- Have identified several additional polymorphic genetic markers associated with smoking and other "risk-taking" behaviors. These have been characterized and optimal conditions for PCR analysis have been determined.
- Have undertaken greater involvement in the NNDC Resident projects evaluating the role of bone morphogenetic protein (BMP) receptors in bone regeneration in periodontal tissues. Oligonucleotides for use in the assessment of levels of BMP gene expression have been identified and synthesized. Determination of the optimal conditions for PCR using these oligonucleotides is in progress.

Miller

- Relative to the program entitled "Biomarkers for Oral Cancer," DNA isolated from a group of 150 tissue sections obtained from subjects from Puerto Rico has been tested for a variety of HPV genes using PCR procedures. Scoring has begun and dot-blot



hybridizations are being completed. Some recently developed problems with hybridization need to be resolved. Tissue samples from our Greece study have been blocked in paraffin and await sectioning and DNA extraction.

- Relative to the project "Changes in Immunoglobulins as a Result of Smoking Cessation and Relation to Neurotransmitter Genes" funded by NIDR/NIH and jointly conducted by the Navy, Geo-Centers, NIDR, and individuals at the Jerry L. Pettis VA Medical Center in Loma Linda, CA., initial blood and serum samples have been received from Loma Linda. A total of 60 serum samples including initial and final blood draws have now been completed and 150 of a total of 300 subjects have been entered into the study.
- Relative to the project concerning the evaluation of cytokine production by oral fibroblasts, preliminary evaluation of reference markers (actin and cyclophyline) has been completed. In addition, a variety of cDNA's have been obtained from RNA isolated from gingival fibroblasts, pulpal fibroblasts and endothelial cells stimulated in culture with a variety of stimulators (TNF, growth factors, and bacterial components).
- Relative to the project "Characterization of Bone Morphogenetic Protein Receptors in Oral Tissues" final approval from the NNMC Institutional Review Board and Human Use has been obtained. Most of the required PCR primers have been synthesized and initial optimization has been completed for the BMP ALK-6 receptor rt-PCR procedure.
- A proposal to evaluate hypersensitivity and cytotoxic effects of soft denture liners has been submitted for approval from the NNMC Institutional Review Board and Human Use.
- An updated text has been completed for use in teaching Oral Microbiology to Dental Residents and the course has begun.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Beck

- Main objective of this upcoming quarter is to continue with NIDR Epidemiology projects.
- Grow and maintain various fibroblast cell lines for the purpose of identifying the RNA messages by the *in situ* hybridization method. *In situ* hybridization method is useful when looking for a specific gene activation via mRNA production within individual cells of a tissue.



- Begin optimizing the PCR conditions for the bone morphogenic protein receptor primers.

Jones

- Relative to the program entitled "Biomarkers for Oral cancer" for the Puerto Rico study subgroup, it is anticipated that SSCP analysis exons 7 and 9 of the p53 gene will be completed and that DNA sequence analysis will begin.
- Clearance for the expanded genetic analysis of the case-control samples from the Puerto Rico Oral Cancer Study was obtained. Will begin characterization of a number of polymorphic risk-associated genes for these samples.
- As additional patient-derived samples become available, anticipate expanded involvement in the NNDC Resident projects evaluating the role of bone morphogenetic protein (BMP) receptors in bone regeneration in periodontal tissues.
- Anticipate the continued arrival of specimens from the various sites participating in the VA subgroup of the Biomarkers for Oral cancer study. Will begin the extraction of DNAs from these materials and the analysis of genetic variation.
- DNAs from the Taiwan Nasopharyngeal Carcinoma Study subgroup will be further characterized using additional genetic markers. The arrival of additional DNA samples for this study is anticipated and these will be incorporated into the ongoing studies.
- Will characterize the DNAs from the Taiwan Oral Cancer Case - Control Study subgroup to determine the frequencies of additional polymorphic genes-- especially those associated with high-risk behavior.

Publications:

- Sagawa, M., Jones, J.E., Ebina, M., Jensen, S. and Linnoila, R.I. 1997. Detection of K-ras point mutation by in-situ PCR in cell suspensions: Comparison of the indirect and direct methods. *Lung Cancer* 17(2-3):181-195.

Miller

- Relative to the program entitled "Biomarkers for Oral Cancer," HPV evaluations on DNA isolated from a group of 150 tissue sections obtained from subjects from Puerto Rico will be completed and evaluated against epidemiologic data including smoking incidence. It is anticipated that during the next quarter work will be initiated to evaluate HLA polymorphisms in this group.

- Relative to the project "Changes in Immunoglobulins as a Result of Smoking Cessation and Relation to Neurotransmitter Genes" funded by NIDR/NIH and jointly conducted by the Navy, Geo-Centers, NIDR, and individuals at the Jerry L. Pettis VA Medical Center in Loma Linda, CA., final blood samples will be collected and evaluated. It is anticipated that all samples will have been collected by the end of 1997. In addition, DNA will be extracted from blood samples in order to begin evaluation of dopamine receptor and transporter gene polymorphisms.
- Relative to the project "Characterization of Bone Morphogenetic Protein Receptors in Oral Tissues" evaluation of clinical samples will begin. This will involve the preparation of cDNA from RNA isolated from the tissue samples and completion of optimization of PCR conditions for remaining primer pairs.
- Relative to the project concerning the evaluation of cytokine production by oral fibroblasts, optimization of PCR conditions will continue and procedures for *in-situ* hybridization will be developed.
- A new project concerned with a survey of virus associated with periodontal and periradicular infections will be initiated. PCR based procedures will be used to identify specific viral types. During the next quarter it is anticipated that PCR conditions will be optimized for several viral DNA samples and that evaluation of some of the clinical samples will have commenced.
- It is anticipated that the course "Oral Microbiology" will be completed and that "Oral Immunology" will begin.

Publications:

- Euler, G, M.M. D'Alesandro, Hutter, J., and Miller, G. Interleukin -6 in neutrophils from peripheral blood and inflammatory periradicular tissues . Accepted for publication in The Journal of Endodontics.



V. NMRI TOX/DET Dayton, OH

A. TOXICOLOGICAL STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

Ademujohn

- The purpose of the neurobehavioral laboratory coordinator at NMRI/TD is to provide technical support to various aspects of ongoing on-site projects in neurobehavioral research. During this quarter the coordinator has been and will be involved in neurobehavioral testing for the effects of simulated stress factors relating to the Gulf War Syndrome on animal models via computer-aided qualitative and quantitative methods. The coordinator also supervises animal training protocols for upcoming pharmaceutical exposure studies.

Briggs

- General Manager and Senior Contractor Representative for Geo-Centers, Inc., for the NMRI contract at the Toxicology Detachment (NMRI/TD). He serves as a member of the Executive Steering Committee and performs toxicology research as an Associate Investigator. He is responsible for collaborating Geo-Centers, Inc. resources in support of the toxicology research in support of the NMRI/TD mission. Dr. Briggs functions in response to taskings from the Officer-In-Charge of the Detachment. These duties include assuring compliance with the Quality Management Plan.

Connolly

- Cataloging print and non-print materials for circulation
- Ordering and maintaining serials collection, including claiming missing issues
- Handling reference questions
- Providing interlibrary loan assistance
- Locating needed materials in other libraries
- Preparing book orders
- Preparing journals for binding, and processing bound journals back into library



Horton, Dibley

- Maintain Local Area Network (LAN)
- Maintain and upgrade individual Desktop and Laboratory Computers
- Provide answers, support and expertise in correcting computer problems, including all peripherals attached to these systems
- Continue comprehensive program for maintaining system integration and reliability through back-up procedures, documentation, and redundant systems
- Continue to update information Databases IRIS, Medline, Toxline and Serline
- Organize Media, Manuals and Spare Parts
- Prepare ASDPs for procurement of new computer systems, software and peripherals
- Maintain in-house software and databases

Jung, Narayanan, T.K.

During the past quarter, the work carried out by this group was:

Trimethylolpropane (TMPP) Evaluation

- A paper was written on the results of this study and is being reviewed. HPLC analysis of the levels of amino acids and neuropeptides in TMPP exposed rat brains was begun.

Cell Model Project

- This project is on hold until further funding is received.
- A paper was written and given to Dr. Carpenter for review. DBNP was synthesized and the melting point and HPLC elution pattern checked to verify purity.

General

- Several research grant proposals were written and submitted for review.

Kimmel, Reboulet, Whitehead

- Sr. Scientist II- Inhalation Toxicology
- Scientist II
- Scientist I

Ritchie

- Assistant group leader for the Neurobehavioral Toxicology Group at the Triservice Toxicology consortium and NMRI/TD and as Associate Principal Investigator (API) for all currently funded neurobehavioral toxicology-related work units (FY'97 funding of \$850K).

Smith

- Assist in the research performed at the Navy Medical Research Institute/ Toxicology Detachment (NMRI/TD) which entails the following tasks:
- Conduct/ design /oversee studies addressing Navy related research issues.
- Provide necessary paperwork for the accounting of project funding
- Maintain GLP compliant data books on those studies with which there is personal involvement.
- Submit articles/revisions for publication of project findings to peer-reviewed journals.
- Submission of timely progress reports

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Ademujohn

The major technical objectives for this quarter are as follows

- Rangefinding using operant - trained animals and measuring subsequent stages of diminished capacity
- To compile, catalog and computerize the above mentioned data.
- To train pigeons and rats for problem solving protocols
- To start and complete a drug dose curve on Diphenylhydantoin (DPH) on rats
- To compile and analyze previously collected data from the D-Amphetamine, Diazepam and Haloperidol studies completed.
- To obtain operant testing and training data for animals used in operant exposure testing To organize, catalog and generate computer graphics, cumulatively from the above mentioned data.
- To maintain data for future reference in upcoming publications.
- To be responsible for the procurement and securing of all materials used in testing and training protocols



- Responsible for documenting and maintaining operant weights
- Responsible for writing and procurement of standard operating procedures for pigeon, rabbit and rat training protocols
- Responsible for overseeing daily accurate and detailed entries and updates of all work unit laboratory books.
- Responsible for maintaining quality control assurance for all ongoing experiments and/or protocols with / between work unit P. I.'s and laboratory technicians.

Briggs

- Review resource allocations for effective use of Geo-Centers, Inc resources in support of the mission and strategic planning
- Initiate the reproductive risk assessment plan and established the resources to perform semen evaluation studies
- Continue to integrate the Quality Management Program including Protocol reviews, DBNP study audit and protocol review
- Prepare and present a Poster at the Spring Conference
- Prepare an abstract for presentation at an estrogens in the environment meeting
- Complete the DRAFT Quality Assurance paper
- Respond to queries from the O.I.C. relating to human health risk assessments including developmental and reproductive risks

Connolly

- Catalog materials as received
- Catalog materials not yet cataloged
- Provide library service to the toxicology community at WPAFB
- Continue working on a manual card catalog

Horton, Dibley

- Continue to correct software problems with legacy eyeblink software
- Installation of redundant hardware to allow users to work off-line at off site locations
- Continue developing ADP SOP manual

Jung, Narayanan, T.K.

The objectives for this period were to:

TMPP

- Begin setting up the method of amino acid and neuropeptide analysis by HPLC

DBNP

- Synthesize 2 kg of DBNP and verify its purity

General

- Write several grant proposals and submit them for review

Kimmel, Reboulet, Whitehead

- Continuing development of laboratory facilities as well as budget constraints did not permit experimental (technical) objectives to be scheduled for this period. In addition, lack of a functional respiratory protection program for inhalation exposure personnel tasked to work with lethal concentrations of test materials has hampered research efforts. There are projects that are on hold and which could be completed and are required to finalize revisions to a manuscript for publication.

Ritchie

TMPP Mechanisms of Action: Development of Neurobehavioral Molecularization Techniques (WU.1516):

- To complete publication of a major study evaluating the relative capacities of well known human anticonvulsant agents to prevent or counteract neurotoxicity induced by exposure to low or high doses of trimethylolpropane phosphate.
- To complete a study of repeated exposure to TMPP on acoustic startle, prepulse inhibition and acoustic startle habituation.

Mechanisms Involved with Exposure to Select Neurotoxicants (WU.1712):

- To complete literature search and research planning (in conjunction with Dr. Robert Carpenter)
- to assist in redesign and construction of a 3-chamber whole body inhalation facility in NMRI/TD Room 202/203, required for exposure of rats to jet fuel (facility was disassembled and temporarily relocated).



- to prepare neurobehavioral tests for evaluation of 128 rats following chronic exposure to JP-5, JP-8 or diesel fuel vapors.

Development of the Navy Neuro-Molecular Assessment System (the NTAS) (WU.1713):

- To work with Dr. Jan Lin in development of methods and techniques for hippocampal tissue slice.
- To work with Dr. T.K. Narayanan for development of methods and techniques for cell culturing of neuroblastoma cells.
- To work with Dr. Eldon Smith for development of quantitative techniques for analysis of neuroprotein markers (i.e., cFOS, GFAP) in response to toxic insult.
- To write and submit new Navy Work Unit to continue 1516 research through FY'99.

Neurobehavioral Toxicity Assessment Battery (NTAB):

- To begin testing of rats, following challenge with three dose levels of nine pharmaceutical drugs (i.e., ethanol, diazepam, etc.), using the Porsolt Forced Swim Test, Morris Water Maze, and Navy Roto-Wheel/Accelerod.
- To work with Dr. Alan Nordholm in initial conditioned eyeblink classical conditioning rabbits and human subjects.
- To complete operant training of 21 pigeons to be used in validation of NTAB tests involving visual discrimination, higher cognitive function and physiologic irritancy.
- To complete development of human tests (i.e., operant conditioning, auditory startle, eyeblink classical conditioning, etc.) To be compared to NTAB tests for predictive validation. To work with the Veterans Administration Hospital (Dayton, OH) for use of PTSD patient population for initial NTAB testing.

Comparative Neurobehavioral Toxicity Assessment of Three Hydrocarbon Fuels

- To submit U.S. Army, WPAFB IACUC, and Wright State University seven ACUC animal use protocols.
- To hire two new GEO-CENTERS personnel to assist in animal behavioral evaluations.
- To begin relocation of the NMRI/TD Neurobehavioral Laboratory to the Research Facility of the Veterans' Administration Hospital, Dayton, OH.

Smith

Technical support for the following NMRI/TD projects is to be provided for:

Spectrex Fire Extinguishant (SFE)

- The objective of this research is to evaluate the potential health effects of exposure to the by-products of pyrolyzed SFE. SFE is a fire suppressant and a potential replacement for Halon 1301.

Cardiac Sensitization

- The objective of this research is to develop a model for the determination of cardiac sensitization. These initial studies will set the basic background needed for future studies.

Trimethylopropane phosphate (TMPP)

- The objective of this research is to determine the mechanism of action of TMPP. TMPP is a by-product from the breakdown of synthetic lubricants that produces a neurotoxic response.

Contract Representative on the Safety Policy Committee (Sue Prues)

- The objective of this duty is to act as liaison between the Navy and Geo-Centers personnel in addressing the concerns of workplace related safety issues.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Ademujohn

- Trained and conditioned new and incoming rodent and pigeon groups to protocol adaptation.
- Maintenance of all laboratory work unit notebooks
- Implemented several data methods to compile training data and weight maintenance on the all operants.
- Compiled stock animal drug history logs
- Compiling meeting memorandums for the OIC
- Trained all incoming personnel on standard procedures for lab techniques.
- Trained personnel (in house class) on GLP standards for recording raw data into lab notebooks
- Completed Amphetamine and Diazepam drug dose curves and drug study on rats



GEO CENTERS, INC

Briggs

- Correlated with NMRI/TD management on resource allocation and budget compliance issues
- Initiated setting up reproductive risk assessment laboratory and dermal risk assessment laboratories conducted a hire for an ADP technologist
- Networked with collaborators at the Society of Toxicology meeting and attended technical symposia and presentations
- Prepared an abstract and gained clearance for a poster. Wrote the DRAFT Quality Assurance paper and submitted it for editing by the co-author
- Held meetings with THRU staff members to coordinate research opportunities for future projects

Connolly

- 51 books cataloged and prepared for circulation
- 343 card sets prepared for manual card catalog
- 38 articles obtained from local libraries
- 8 books borrowed from local libraries for customers here
- 9 interlibrary loans obtained
- 1 interlibrary loans provided to another library
- 15 literature searches conducted using in-house CD-ROM database capabilities
- 2 searches successfully conducted on the internet for customers, including downloading of documents as required
- 6 reference questions answered
- 3 telephone inquiries on journal locations in local area handled successfully
- 29 requests for articles located and filled from in house resources
- 4 articles obtained using the CARL UnCover system via the internet
- 3 orientation/training session conducted
- 74 journal volumes consulted by customers

Horton, Dibley

- Deployed new server "Defiant". Just recently received new hardware to stabilize this server and allow it to be fully functional
- Installed new UPS maintenance software that has allowed an accurate measurement and logging of power throughout the building and extremely accurate detection and measurement of power spikes and brown-outs as they occur.



- Avoided network shutdown through software and service reorganization.
- Researched new software that would enhance Virus protection for this command.
New software is being investigated that will work in conjunction with current systems and software that will significantly enhance our virus protection through interception and disinfection of internet and e-mail messages while still in message que - software is called "Mine Sweeper".
- Updated Service Packs and Hot Fixes as needed on Network Servers
- Ordered various software and hardware upgrades
- Continued to reconfigure Windows Browser and WINS for WAN
- Developed method for portable storage of data via a CD/PD writer for Neurobehavior
- Continued maintenance of Servers including backing up data files
- Continued support of hardware and software for TOXDET personnel
- Continued to update information Databases
- Created and implemented a beta version of database to be used by staff Librarian for maintaining inventory and rapid tracking of available resources
- Continued development of ADP SOP manual - this is an ongoing process that will assist NMRI/TD to meet GALP guidelines
- Mr. Horton and Mr. Dibley continue to attend the full MCSE course at Miami-Jacobs College on their own time. Both have completed the Administering NT and Windows NT Core Technologies. In addition, both have successfully completed the Microsoft TCP/IP and Windows NT Server examinations and have become certified Microsoft Product Specialists.

Jung, Narayanan, T.K.

TMPP

- The prepared paper is still in the review process. Ms. Jung began working with Dr. Lindsey on setting up a method of amino acid analysis using a BAS HPLC. At this point in time, the standards are being separated and their elution times verified. An abstract for SOT is being prepared for review.

Cell model

- This project was put on hold until further funding was received. The liver hepatocyte cells were placed in cryostorage until they will be needed.



DBNP

- This project was completed. We synthesized 2.4 kg of DBNP. The purity of the crystals was verified by melting point and HPLC elution pattern data. The paper is still being reviewed.

General

- Dr. Narayanan prepared several research grant proposals.

Kimmel, Reboulet, Whitehead

- Completed installation of several new inhalation exposure systems. Began installation and performance verification of systems and instrumentation to perform small animal pulmonary function tests during and post inhalation exposure. Three basic types of pulmonary plethysmography are to be employed capable of gathering over 100 different indices of pulmonary function. Systems to perform barometric plethysmography in conscious, free roaming animals are functional. Systems to perform flow plethysmography during and after inhalation exposure have been installed, calibrated and function verified. Installation of complete function assessment system is approximately 50% complete. Protocols have been submitted for two initial experiments utilizing these systems. Progress on the work units which these systems support is on schedule.
- Several iterations of computer model of aerosol deposition in rats and in humans based on respiratory system structure and function as well as aerosol physics and airways fluid dynamics have been completed, work is in progress to verify the model against deposition curves reported in the literature. (For risk assessment and publication). First drafts of a manuscript are in progress. EC Kimmel, RL Carpenter.
- Reviewed editors comments and resubmitted a revised manuscript to Am. Ind. Hyg. Assoc. J. Entitled "Calculation of exposure chamber leak rate with thermal correction: A measure of chamber integrity". By EC Kimmel and JE Reboulet
- Presented "Concepts in Inhalation Toxicology of Fire Suppressants: Pneumotoxicity" at the Halon Options Technical Working Conference, in May, Albuquerque, NM.
- Submitted an abstract for presentation at 1998 SOT meeting entitled "A model for predicting carboxyhemoglobin formation rate in F-344 rats" by EC Kimmel, EA Smith, JE Reboulet and RL Carpenter.
- Submitted an abstract to 1998 SOT entitled "Use of an aerosol deposition model to compare inhalation toxicity risk between laboratory and field aerosol exposures." RL Carpenter and EC Kimmel.



- Submitted a Letter of Intent (pre-proposal) to combined DoD/VA funding source entitled "Military operational stress-related illness, pulmonary chemical hypersensitization: Physiological markers of sensitization."
- Tentative approval for respiratory protection program for the inhalation laboratories has been given.
- Wrote a final draft of a CRADA for collaborative research with Dr. C. Gairola (pulmonary biochemist) of the University of Kentucky. This CRADA has been through Navy approval process and is now in the hands of the University of Kentucky for final modifications.

Ritchie

TMPP Mechanisms of Action: Development of Neurobehavioral Molecularization Techniques (WU.1516)

- Began data analysis and publication of study evaluating the relative capacities of well known human anticonvulsant agents (valproic acid, ethosuximide, diazepam and phenobarbital), combined with GABA_B antagonist to prevent or counteract absence-like seizures induced by exposure to doses of trimethylolpropane phosphate.
- Begin analysis of 15 rats with bipolar electrodes in the nucleus accumbens or ventral tegmental area for evaluation of TMPP-induced effects on intracranial self-stimulation.
- Completed study of the effects of repeated TMPP exposure on acoustic startle response, prepulse inhibition and acoustic startle habituation.
- Completed study of the effects of TMPP on eyeblink classical conditioning in the rabbit.
- Submitted four abstracts for the Society of Toxicology Meeting, 1998.
- Submitted new Navy Work Unit proposal for funding FY'98-99,

Mechanisms Involved with Exposure to Select Neurotoxicants (WU.1712)

- Completed literature survey, emphasizing neurobehavioral consequences of exposure of animals or humans to two levels of jet fuels, gasoline and kerosene.
- Assisted in re-engineering to reconstruct a whole body inhalation facility in NMRI/TD Room 202/203.
- Submitted new Navy Work Unit proposal.
- Began pilot studies for the microdialysis monitoring of TMPP distribution throughout the CNS.



Development of the Navy Neuro-Molecular Assessment System (the NTAS) (WU.1713)

- Assisted in completion of hippocampal tissue slice methods.
- Assisted in successful cell culturing of neuroblastoma cells.
- Assisted in ordering of supplies for neuroprotein analysis.

Neurobehavioral Toxicity Assessment Battery (NTAB)

- Completed studies compaing dose response effects of ethanol, amphetamine and diazepam on Porsolt FST, operant progressive ratio and Navy Roto-Wheel performances in rats.
- Developed testing methods for conditioned eyeblink classical conditioning of rats, rabbits and human subjects.
- Completed initial validation of the NTAB using operant training of 21 pigeons.
- Completed initial planning for NTAB testing of human subjects at : (a) the VA Hospital, Dayton, OH; (b) two remote Naval stations; and (c)WPAFB.

Comparative Neurobehavioral Toxicity Assessment of Three Hydrocarbon Fuels

- Submitted the U.S. Army protocol, WPAFB IACUC animal use protocol, and Wright State ACUC protocol related to the fuel oil exposure study.
- Finalized relocation of hte NMRI/TD Neurobehavioral Laboratory to the Research Facility of the Veterans' Administration Hospital, Dayton, OH; expected to occure Oct 1, 1997.

Publications

- Lin, J., Cassell, J., Ritchie, G.D., and Rossi III, J. Repeated exposure to trimethylolpropane phosphate induces cns sensitization and facilitates electrical kindling. *Physiology and Behavior*, submitted for Publication, 1997.
- Rossi III, J., Nordholm, A.F., Ritchie, G.D and Still, K.R. Neurobehavioral thrust area for the deployment toxicology strategic plan, FY 1998-2028. *Position Paper*, Tri-Service Toxicology Consortium, 1997.
- Lindsey, J.W., Prues, S.L, Alva, C., Ritchie, G.D. and Rossi III, J. Trimethylolpropane phosphate microperfusion into the nucleus accumbens of rat: electroencephalic, behavioral and neurochemical correlates. Accepted for publication, *NeuroToxicology*, 1997.



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- Rossi III, J., Narayanan, T.K., Jung, A., Nordholm, A.F. and Ritchie, G.D. Effects of PTZ- or TMPP-induced generalized seizures on neurotransmitter and metabolite levels in five brain regions. *Abstract*, Society of Toxicology, Annual Meeting, Seattle, WA, 1998.
- Rossi III, J., Walter, M., Bekkedal, M.Y.V., Borkowski, C.M., Ritchie, G.D., Nordholm, A.F. and Panksepp, J. Developmental neurobehavioral effects of prenatal and early postnatal exposure to trimethylolpropane phosphate (TMPP), *Abstract*, Society of Toxicology, Annual Meeting, Seattle, WA, 1998.
- Ritchie, G.D., Hulme, M.B., Nordholm, A.F. and Rossi III, J. Effects of GABA_B antagonist CGP-35348 and human anti-epileptic drugs on spontaneous and chemically-induced absence-like SDWs in Fischer-344 rats.
- Lin, J., Cassell, J., Ritchie, G.D. and Rossi III, J. Repeated exposure to trimethylolpropane phosphate (TMPP) facilitates electrical kindling of the amygdaloid complex in male rats. *Poster Presentation*, Society for Neuroscience Annual Meeting, New Orleans, LA, Oct 1997.
- Rossi III, J., Narayanan, T.K., Jung, A. and Ritchie, G.D. Effects of PTZ- or TMPP-induced generalized seizures on neurotransmitter and metabolite levels in five brain regions. *Poster Presentation*, Society for Neuroscience Annual Meeting, New Orleans, LA, Oct 1997.
- Nordholm, A.F., Rossi III, J., MacMahon, K. and Ritchie, G.D. Acute and long-term consequences of repeated exposure of rats to JP-4 jet fuel and/or stress. *Poster Presentation*, Society for Neuroscience Annual Meeting, New Orleans, LA, Oct 1997.
- Ritchie, G.D., Rossi III, J., Hulme, M.B., Ademujohn, C.Y. and Cassell, J. Effects of GABA_B antagonist CGP-35348 and human anti-epileptic drugs on spontaneous and chemically-induced absence-like SDWs in Fischer-344 rats. *Poster Presentation*, Society for Neuroscience Annual Meeting, New Orleans, LA, Oct 1997.



- Rossi III, J., Nordholm, A.F. and Ritchie, G.D. MILITARY DEPLOYMENT TOXICOLOGY: PREDICTIVE NEUROBEHAVIORAL ASSESSMENT STRATEGIES. *Poster Presentation*, Behavioral Toxicology Society Annual Meeting, W. Palm Beach, FL, June 1997.

Smith

SFE Project -- Homeostasis and Edema

- Conducted baseline blood gas studies to ascertain any homeostatic effects following a serial blood collection/transfusion.
- Prepared abstract for submission of a poster on the Transfusion study data to be presented at the Annual meeting of the Society of Toxicology to be held in Seattle, Washington in March of 1998

Cardiac Sensitization

- Continue probing the mechanical and electrophysiological events leading to ventricular fibrillation using the swine model.

TMPP Project -- Glial Fibrillary Acidic Protein (GFAP)

- Startup preparation of an ELISA assay method for quantitative determination of GFAP within brain tissue.
- Provide assistance/training for projects requiring drug preparation, surgical implantation of devices via stereotaxic methodology, etc. as needed.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Ademujohn

- To accurately oversee the training of rodents for various testing protocols, such as EEG, swimtest..
- To oversee rabbit testing, training and conditioning for upcoming neurobehavioral studies.
- To maintain a clean and orderly laboratory environment.
- To provide technical support in testing relative toxicity of various pharmaceuticals in pigeons and rats and rabbits.
- To procure and document pigeon maintenance pertaining to preparatory requirements for 'shaping' activities , pre-testing and testing protocols.



GEO CENTERS, INC.

- Maintain quality assurance in all levels of data acquisition, processing and retrieval for all completed and ongoing lab experiments and protocols.
- To compile and organize the raw laboratory data into a centralized GLP standard retrieval system
- To start and complete drug dose curve/ study on haloperidol, diphenylhydantoin, and scopolamine.
- To publish results of above mentioned drug study.

Presentations :

- Ritchie, G.D., Rossi III, J., Hulme, M.B., Ademujohn, C.Y. and Cassell, J. Effects of GABA_B antagonist CGP-35348 and human anti-epileptic drugs on spontaneous and chemically induced absence-like SDW's in Fischer-344 rats. *Abstract*, Society for Neuroscience Annual Meeting, New Orleans, LA, Oct 1997.

Briggs

- Complete the staffing and resource allocation process and assign Geo-Centers staff to projects and support services
- Perform preliminary studies in rabbits to assess the potential for military relevant chemicals to produce human health risks in a surrogate laboratory animal model
- Assisted in supporting the ARDS and Cardiac Sensitization projects and helped to correlate these functions into the Deployment Toxicology Program
- Continued to perform planning and technical support to NMRI/TD management as tasked

Connolly

- Continue cataloging
- Continue preparing cards for the manual card catalog
- Continue training program

Horton, Dibley

- Finalize the ADP SOP manual
- Perform desktop software inventory to check for licensing problems
- Bring Excalibur server on-line



- Provide guidelines for installation of network resources for the Neurobehavior group at the VA lab once the plan for the network is delivered.
- Mr. Horton should attend a comprehensive Network Security course
- Mr. Dibley should attend an in-depth Microsoft Exchange course

Jung, Narayanan, T.K.

- Increase the productivity in the lab
- Continue the TMPP binding studies on the benzodiazapine receptor using ^{35}S and ^{36}Cl .
- Continue work on the brain amino acid content analysis
- Begin a study on the enzymes glutamic acid decarboxylase and GABA transaminase, serine -trans-hydroxymethylase, aspartate aminotransferase, and glutamine oxidase.
- Begin a study based upon the four endocrine preproposals written earlier.
- "Absorption, Distribution, Metabolism, and Excretion of 2,6-Di-Tertiary-Butyl-4-Nitrophenol in Fischer-344 Rats" TK Narayanan, A. E. Jung, S. L. Prues, R. L. Carpenter and K. R. Still
- "Tissue Distribution, Metabolism, and Clearance of Trimethylolpropanephosphate (TMPP) in Fischer-344 Rats: Tanjore K. Narayanan, Anne E. Jung, Glenn D. Ritchie, John F. Wyman, and John Rossi III.
- "Acute Effects of a Bicyclicophosphate Neuroconvulsant on Monoamine Neurotransmitter and metabolite Levels in the Rat Brain" James W. Lindsey, Anne E. Jung, Tanjore K. Narayanan, Glenn D. Ritchie.
- Research Proposal: "Signal Transduction Mechanisms in Cardiac Toxicity by halogenated hydrocarbons. 3 years- \$409K.

Pre-proposals:

- Monoamines, Glucocorticoids and corticotropin releasing factors in prolonged stress. Modulation of neuro-endocrine response due to stress by cytokines.
- Phenotypic and neurotypic markers for neurotoxicity by environmental factors.
- Oxidative stress-mediated calcium deregulation as a common mechanism in neuronal celldeath.

Kimmel, Reboulet, Whitehead

- Complete installation of first stage (fundamental measurements ventilation, respiratory mechanics, and gas exchange - 100% if oaraneter determination) of small animal pulmonary function testing laboratory. Perform pfts on rats and guinea pigs



for the purpose of refinement of technique and establishment of historical control values for over 100 indices of pulmonary function.

- At present the main barrier to any further progress is the lack of a respiratory protection program for workers in the inhalation toxicology group.
- Conduct two experiments: 1. Verification of a mathematical (first principle) model of carboxyhemoglobin formation by CO exposure in small animals. 2. Development of and verification of a mathematical (first principle) model of stimulation of ventilation in small animals by exposure to CO₂.
- Finish first draft of a manuscript entitled "augmentation of acrolein induced acute lung injury by aerosol co-exposure" by EC Kimmel, JE Reboulet, GS Whitehead, and RL Carpenter.
- Establish a working relationship with Wright State University to obtain histopathology services to support our research efforts.

Ritchie

TMPP Mechanisms of Action: Development of Neurobehavioral Molecularization Techniques (WU.1516)

- To complete replication of study investigating effects of repeated exposure to low doses of TMPP on acoustic startle in rats; abegin study publication.
- To complete publication of study investigating effects of AEDs and a novel GABA-B antagonist on TMPP-induced paroxysms.
- To begin study investigating effects of TMPP on eyeblink classical conditioning in rats.

Mechanisms Involved with Exposure to Select Neurotoxicants (WU.1712)

- Work Unit funding ends 30 September 1997.

Development of the Navy Neuro-Molecular Assessment System (the NTAS) (WU.1713)

- To assist in completion of initial studies of hippocampal tissue slice analysis of TMPP exposure, effects of TMPP exposure of CNS tissue culture electrophysiology, and neuroprotein analysis of TMPP-induced neurotoxicity.

Neurobehavioral Toxicity Assessment Battery (NTAB)

- Continue study of the effects of dosing (three dose levels) with one of nine pharmaceutical drugs on behavior of rats, rabbits or pigeons on each of seven NTAB neurobehavioral tests.



Comparative Neurobehavioral Toxicity Assessment of Three Hydrocarbon Fuels

- Complete initial oral and dermal exposure of 132 rats to JP-8 fuel; neurobehavioral evaluation of exposed rats.

Smith

- Surgical and technical support for the Homeostasis portion of the SFE project.
- Ongoing support/development of a GFAP immunoassay (to investigate subtle changes in the central nervous system) for the TMPP project
- Continue representation of Geo-Centers concerns regarding health and safety.
- Conduct/support pulmonary physiology studies with regard to aerosol effects on the lung.
- Continue writing new protocols in the area of pulmonary toxicology.
- Close data books for SFE range-finding/multiple-dose, edema and blood gas studies..

Publications

- Eldon A. Smith, Edgar C. Kimmel, Larry E. Bowen, James E. Reboulet, and Robert L. Carpenter, Preliminary Assessment of a Pyrotechnically Generated Aerosol Fire Suppressant. *Inhalation Toxicology*, (1997) 9:449-463.
- Eldon A. Smith, Edgar C. Kimmel, Jeffery H. English, Larry E. Bowen, James E. Reboulet, and Robert L. Carpenter, Evaluation of the Respiratory Tract after Acute Exposure to a Pyrotechnically Generated Aerosol Fire Suppressant. *Journal of Applied Toxicology*, (1997) 17(2):95-103.
- Eldon A. Smith, Edgar C. Kimmel, James E. Reboulet, Susan L. Prues, and Robert L. Carpenter, Comparison of Toxicity after Exposure to Two Formulations of SFE Formulation A, *Fundamental and Applied Toxicology* (in review).



V. NMRI, Natick, MA

A. HUMAN PERFORMANCE AND U.S. NAVY CLOTHING DEVELOPMENT

DESCRIPTION OF WORK TO BE PERFORMED

Pawar

Program I: Flame Protective Clothing Research

- The primary research goal for the current reporting period was to complete calibration routines for the automation of Thermal Protection Performance (TPP) equipment, demonstrate the Burn Injury Sensor Calibration System (BISC) for its usefulness in selection of sensor to suit given fire hazard and validate the Wissler math model for additional data on rough and calm seas. However, due to the high priority given to completion of math modeling project the TPP sensor calibration and development of the BISC system could not proceed as planned. Therefore, major part of this quarter was spent on Math Modeling Project which is completed to a stage of writing final report.

Macek

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment

- GEO-CENTERS, INC. will establish a program to be used by NCTRF to certify commercial off-the-shelf protective clothing/equipment as meeting or exceeding Navy functional performance requirements. This program will make possible the direct purchase of certified commercial protective clothing/equipment for shipboard use by Navy personnel.

Macek, Collins

Program III: Database Search

- Conduct an extensive search of databases to determine commercial, DoD and non-DoD government organizations with which the U.S. Navy Clothing & Textile Facility (NCTRF) may enter into cooperative R&D agreements for the research, development, and testing of dress and protective clothing systems.



GEO CENTERS, INC.

- Determine cooperative opportunities for dual-use technology, technology transition, and technology exploitation.
- Prepare a technical briefing to highlight the technical expertise and unique facilities and equipment available at NCTRF. This briefing could be used by agencies seeking cooperative research, development, and acquisition agreements.
- Prepare documentation to convey the technical expertise and unique facilities and equipment available at NCTRF. This documentation could be used by NCTRF employees to serve as a marketing tool and as a handout after the technical briefing is presented.

Buller, Collins

Program IV: Great Lakes Prototype Footwear Test

- Provide technical support in the development of the Enhanced Chukka Shoe surveys for recruits, leaders, shipboard personnel, and Naval Academy personnel.
- Provide technical support for experimental design of study.
- Provide software support in the production of an on-line data entry program and database management.
- Provide data collection support at the Recruit Training Center (RTC).
- Analyze data by test group and write final report of findings of the study.

Macek, Schneider

Program V: Technical Reports

- Analyze and organize information provided on projects conducted in the Navy Clothing and Textile Research Facility (NCTRF).
- Develop technical reports and articles for publication in peer-reviewed journals.



Buller, Meyers, Collins

Program VI: Utility Uniform Study

Commercial-Off-the-Shelf Utility Uniform Study

- Design questionnaire to assess fit, performance, durability and preference for two commercial off- the-shelf utility uniforms. The two styles are: 1) Redcap, and 2) Levi 505.
- Produce issue data sheets and explanatory package for subjects.
- Reproduce questionnaires and issue packages.
- Analyze data.
- Write final report.

Main Utility Uniform Study

- Adapt questionnaire, data sheets, and explanatory package from COTS study for three uniform configurations: 1) 14 oz. Denim with 4 oz. Chambray Shirt, 2) 11 oz. Denim with 4 oz. Chambray Shirt, and 3) "Dickie" Style.
- Reproduce questionnaires and issue packages for all test participants.
- Provide support of two issuers to 16 test sites on the East and West Coasts, with approximately 75 test participants at each site.
- Provide support of two Human Factors Engineers to visit each test site twice during the duration of the study to issue and collect surveys and to collect subject comments. Visits will occur three and six months after issue of utility uniforms.
- Enter, clean, verify, and tabulate collected data.
- Analyze data based upon experimental design and study hypothesis, using standard univariate and multivariate statistical techniques.
- Write report detailing whole study providing a clear explanation of the analytical techniques adopted and the conclusions reached from analysis of the data.



Buller, Stern-Wolfson

Program VII: Oxford Shoe Study

- Design questionnaire to assess fit, performance, durability and preference for three Oxford shoe sole configurations.
- Design issue data sheets.
- Enter, clean, verify, and tabulate collected data.
- Analyze data based upon experimental design and study hypothesis, using standard univariate and multivariate statistical techniques.
- Write report detailing whole study providing a clear explanation of the analytical techniques adopted and the conclusions reached from analysis of the data.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Pawar

Program I: Flame Protective Clothing Research

- To complete the final report for this task.

Macek

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment

- Complete editorial changes to the certification program report and submit to NCTRF for review and comment.

Macek, Collins

Program III: Database Search

- Await results of final review of the prototype brochure, folder cover, and information sheets.

Buller, Collins

Program IV: Great Lakes Prototype Footwear Test

- None.

Macek, Schneider

Program V: Technical Reports

- To conduct work on two reports for the Navy Clothing and Textile Research Facility.

Buller, Meyers, Collins

Program VI: Utility Uniform Study

COTS Study

- Analyze and produce summary report of the Midpoint survey data.

Main Utility Uniform Study

- Enter, clean and verify all "Issue" and "Midpoint" data.
- Analyze combined "Issue" and "Midpoint" data set.
- Report analysis of data in summary format.

Buller, Stern-Wolfson

Program VII: Oxford Shoe Study

- Enter, verify, and clean all issue and phase one data.



SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Pawar

Program I: Flame Protective Clothing Research

- Complete and finalize writing of the final report.

Macek

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment

- None.

Macek, Collins

Program III: Database Search

- Awaiting results of final review of the brochure, folder cover, and information sheets.

Buller, Collins

Program IV: Great Lakes Protective Footwear Test

- None.

Macek, Schneider

Program V: Technical Reports

- Work was conducted on a report which dealt with the development of a laboratory method of rough sea simulation for the immersion testing of protective clothing using a Wave Maker that was installed in the NCTRF environmental tank. Measurements were made of body cooling rates, both rectal and skin temperatures, as well as heat loss. Comparisons were made between the body cooling rates in successive wave maker trials and with the results of both calm water conditions and the body cooling rates



experienced in earlier trials in the field at Cape May, NJ. A draft of the technical report, titled *Comparison of Field and Laboratory Tests of Body Cooling Rates Using a Wave Maker to Simulate Rough Seas*, has undergone full editorial review.

- The second report which was worked on is a Report of Invention (the first step in seeking a patent). The invention deals with a method of protecting the hands and other extremities from frostbite by extracting heat from warmer parts of the body and using a pumped fluid to transfer the heat to the chilled extremities. The use of the body as a source of heat and a pumped fluid for heat transfer overcomes the limitation of alternatives, such as a battery source of power for electrically-heated gloves or the use of heat pipes for body heat transfer. The full text of the report of invention has been developed with the title, *Pumped Fluid System for Body Heat Transfer*. Some references, to be supplied by the Project Officer are needed to complete the article.

Buller, Meyers, Collins

Program VI: Utility Uniform Study

COTS Study

- All "Midpoint" data were entered by NCTRF personnel according to the coding system designed in the questionnaires. These data were analyzed using standard multivariate and nonparametric techniques. The results of these analyses were summarized and reported.

Main Utility Uniform Study

- All "Issue", "Midpoint" and part of the "Final" data were entered, verified, and cleaned.
- All "Midpoint" data and "Issue" data were combined and analyzed using multivariate, univariate, and nonparametric procedures. The results of this analysis were reported in a summary format.
- All available "Final Phase Data" were entered, verified, and cleaned.

Buller, Stern-Wolfson

Program VII: Oxford Shoe Study

- All issue and phase one data were entered, verified, and cleaned.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Pawar

Program I: Flame Protective Clothing Research

- None.

Macek

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment

- Upon receiving comments from NCTRF on the certification program report, GEO-CENTERS, INC. will incorporate the changes into the report.

Macek, Collins

Program III: Database Search

- Revise draft brochure, folder cover, and information sheets in accordance with suggested changes obtained from NCTRF.
- Deliver electronic files for use to produce high quality brochures, folder covers, and information sheets.
- Close out task.

Buller, Collins

Program IV: Great Lakes Protective Footwear Test

- None

Macek, Schneider

Program V: Technical Reports

- Complete work on the two projects described above and submit the draft technical reports to the Project Officers for review.
- Begin work on an article dealing with the NCTRF studies of laboratory rough sea simulation methods for publication in a peer-reviewed technical journal.

Buller, Meyers, Collins

Program VI: Utility Uniform Study

COTS Study

- Analyze all data and write a final technical report.

Main Utility Uniform Study

- Enter, clean, and verify all "End-Point" data.
- Analyze all combined data.
- Write final technical report.

Buller, Stern-Wolfson

Program VII: Oxford Shoe Study

- Enter, verify and clean, phase two and phase three data.
- Analyze combined data set.
- Provide summary report of combined data.



GEO CENTERS, INC.